Metabolic reprogramming of murine cardiomyocytes during autophagy requires the extracellular nutrient sensor, decorin

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Running title: Decorin senses nutrient status for proper cardiac function

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Keywords: decorin, autophagy, proteoglycan, extracellular matrix, metabolomics, heart, O-GlcNAcylation, fasting, cardiac function, nutrient sensing

ABSTRACT

The extracellular matrix is a master regulator of tissue homeostasis in health and disease. Here we examined how the small, leucine-rich extracellular matrix proteoglycan, decorin, regulates cardiomyocyte metabolism during fasting in vivo. First, we validated in Dcn⁻/⁻ mice that decorin plays an essential role in autophagy induced by fasting. High-throughput metabolomics analyses of cardiac tissue in Dcn⁻/⁻ mice subjected to fasting revealed striking differences in the hexosamine biosynthetic pathway resulting in aberrant cardiac O-β-N-acetylglycosylation as compared to wild-type mice. Functionally, Dcn⁻/⁻ mice maintained cardiac function at a level comparable to non-fasted animals whereas fasted wild-type mice showed reduced ejection fraction. Collectively, our results suggest that reduced sensing of nutrient deprivation in the absence of decorin preempts functional adjustments of cardiac output associated with metabolic reprogramming.

Decorin, a small, leucine-rich proteoglycan (SLRP) localized to the extracellular matrix (1-3), regulates numerous functions to maintain cellular homeostasis as well as to prevent tumorigenesis (4,5), making it a bona fide “guardian from the matrix” (6). Most recently, the research surrounding decorin has involved studying its increasingly important role in the control of catabolism. Specifically, decorin initiates autophagy in endothelial (7,8) and glioma cells (9) and mitophagy in triple-negative breast carcinoma cells (10) via its interaction with and signaling through receptor tyrosine kinases (RTKs). Furthermore, decorin is itself an autophagy-sensitive factor (11) where it is induced in response to nutrient deprivation as well as following direct mTOR inhibition. Additionally, we discovered that mice lacking decorin are insensitive to starvation-induced cardiac autophagy following a one-day period of fasting (11).

In the context of metabolism and metabolic disorders, nutrient and energy status are emerging as intimate partners with autophagy and its deregulation. In particular, research in this area illustrates that abnormal glucose metabolism results in augmented flux through the hexosamine biosynthetic pathway (HBP), leading to increased levels of protein O-GlcNAcylation and consequent inhibition of autophagy (12,13). Taken together, as aberrations in both autophagy and metabolism have been implicated in many cardiac disorders, we...
questioned whether decorin plays a role in linking these processes with cardiac function.

In this study, we show that decorin is a crucial nutrient sensor in vivo that is required for the induction of fasting-mediated cardiac autophagy, a fundamental process that has been shown to be cardioprotective (14,15). Moreover, we show that Dcn$^{-/-}$ mice differed from wild-type mice in their cardiac glucose utilization, subsequently resulting in anomalous O-GlcNAcylation following nutrient-related stress. We discovered that these differences in autophagy and metabolism altered cardiac function as genetic ablation of decorin preserved ejection fraction following fasting, and this could be reversed by systemic delivery of recombinant decorin. Thus, we present a new role for an extracellular matrix proteoglycan at the epicenter of autophagy and metabolism, which modulates cardiac function. These results contribute to a better understanding of how factors outside the cell are imperative for regulating intracellular processes leading to physiologic consequences. Hence, we propose that these findings will pave the way for other discoveries of outside-in signaling that will enhance our ability to regulate biochemical processes in a manner that will ultimately be useful in a translational setting.

RESULTS

Prolonged fasting cannot transcend the cardiac autophagic defect in Dcn$^{-/-}$ mice

As our previous work demonstrated the necessity of decorin expression for cardiac autophagy in response to 25 h nutrient deprivation ((11) and Fig. S1), we investigated the possibility that prolonged fasting (48 h) could overcome this autophagic impairment in Dcn$^{-/-}$ mice. To this end, we evaluated expression of the lipidated version of microtubule-associated light chain protein 3 (LC3-II). Since this lipidated form of LC3 associates with the autophagosomal membrane, quantification of its levels are often used as an accurate approximation of autophagic activity (16). Interestingly, in wild-type mice, we found that a 48-h fasting resulted in robust conversion of cardiac LC3-I to LC3-II, indicating enhanced autophagic activity, whereas, under the same conditions of nutrient deprivation, LC3-II levels were unchanged in the Dcn$^{-/-}$ hearts (Fig. 1A). Indeed, 48 h-fasted levels of cardiac LC3-II were significantly lower in Dcn$^{-/-}$ vis-à-vis wild-type mice (Fig. 1B), illustrating that their autophagic capability in response to nutrient deprivation remained impaired even with protracted stimulation of this catabolic pathway. We were curious as to the blunted autophagic response to starvation in the Dcn$^{-/-}$ heart, and so we delved deeper into the nuances of decorin-mediated cardiac autophagy and found that Dcn$^{-/-}$ mice were less sensitive to mTOR inhibition than their wild-type equivalents. Specifically, we noted lower levels of LC3-II following Torin 1 administration in mice lacking decorin than in wild-type controls (Fig. S2A and S2B). Moreover, after blocking autophagic flux in vivo via chloroquine administration, fasted Dcn$^{-/-}$ mice exhibited lower cardiac LC3-II levels than fasted Dcn$^{+/+}$ mice, further supporting the idea that Dcn$^{-/-}$ hearts undergo lower levels of autophagy than Dcn$^{+/+}$ mice (Fig. S2C and S2D). In addition, we examined the levels of another autophagic effector, Beclin 1 and found, as others have (17), that fasting did not change appreciably the levels of Beclin 1 in wild-type hearts, nor were Beclin 1 levels significantly altered between the two genotypes in either the fed or the fasted state (Fig. S2E and S2F).

We also recognized that our model relied on whole cardiac tissue lysates. To determine which cell types were most affected by lack of decorin, we localized LC3 in cardiac tissue sections primarily to cardiomyocytes. As these cell types make up the majority of the composition of the heart, we concluded that the autophagic differences (quantified here via analysis of LC3-positive puncta) between wild-type and Dcn$^{-/-}$ hearts were potentially caused by the effects of circulating decorin on these cardiac myocytes (Fig. 1C, 1D, 1E, 1F, and 1G). Thus, we hypothesized that decorin acts as an essential nutrient sensor in vivo to provoke this catabolic process in cardiac tissue when energy levels are low.

Decorin treatment rescues cardiac autophagy levels in response to nutrient deprivation

Of note, the protein core of decorin interacts with RTKs to modulate intracellular signaling cascades (18-23) while the glycosaminoglycan chain is dispensable for most of its biological activity (24,25). This finding is especially true for its ability to evoke autophagy as we have previously described (7). Therefore, to test our hypothesis that decorin is a nutrient sensor in vivo, we treated Dcn$^{-/-}$ mice with recombinant human decorin protein.
core (10 mg/kg) and then assessed autophagy levels following 25 h of fasting. We first examined expression of the Map1lc3a gene encoding LC3, since this gene is inducible by starvation in wild-type, but not Dcn−/− hearts (11). Remarkably, treatment with exogenous decorin resulted in fasting-mediated induction of this gene in Dcn−/− cardiac tissue, though to levels lower than wild-type counterparts (Fig. 1H). More importantly, decorin treatment caused an increase in LC3-II protein levels in fasted Dcn−/− hearts that was comparable to the levels observed in wild-type mice following this same period of nutrient deprivation (Fig. 1I and 1J). Therefore, introducing decorin into mice devoid of this proteoglycan rescues the fasting-related autophagic defect in the heart. Interestingly, decorin treatment did not alter levels of LC3-II in the fed state of Dcn−/− hearts compared to fed wild-type levels (Fig. 1I and 1J). Thus, the pro-autophagic effects of decorin must occur primarily in a nutrient-depleted state, confirming a new role for this SLRP as a cardiac nutrient sensor to regulate catabolism.

As decorin is a well-documented pan-RTK inhibitor (5), we surmised that decorin’s nutritionsensing ability must transpire via an outside-in receptor-mediated pathway. Given that the insulin-like growth factor 1 receptor (Igf1r) is a known decorin-binding partner (19,25,26) and because Igf1r signaling is vital for both metabolic homeostasis and cardiac function (27), we explored the possibility that Dcn−/− mice display dysregulated signaling through this RTK. While we found similar expression of total cardiac Igf1r between wild-type and Dcn−/− mice in both fed and fasted states, we found differential phosphorylation of this receptor at the Tyr1135/36 residues (Fig. 1K and 1L). Specifically, there were relatively high levels of Igf1r phosphorylation in the fed state in wild-type hearts, which were reduced significantly upon 25-h fasting (Figures 1K and 1L). In contrast, phosphorylated Igf1r in Dcn−/− mice was significantly lower in the fed state and remained low following fasting (Figures 1K and 1L). This dichotomy in Igf1r phosphorylation status correlated with the relative insensitivity to nutrient-related cues in the absence of decorin. Moreover, combining this aberrant Igf1r phosphorylation with the autophagic deficiency in Dcn−/− hearts suggests potential metabolic irregularities in these mice.

Dcn−/− mice exhibit global metabolism comparable to wild-type mice

Given these findings, we compared common metabolic parameters in wild-type and Dcn−/− mice to determine whether systemic differences in metabolism might explain the suppressed autophagy in Dcn−/− hearts. We began by measuring blood glucose in the fed, 25 h-fasted, and 48 h-fasted states. We chose a consistent time of day (morning) to measure the glucose levels among many mice over multiple experiments to ensure scientifically robust and accurate recordings, especially for the non-fasted animals. Surprisingly, Dcn−/− mice had elevated fed blood glucose levels vis-à-vis wild-type mice (Fig. 2A). However, both standard (25 h) and extended (48 h) periods of fasting resulted in similar levels of hypoglycemia in both genotypes (Fig. 2A). Despite the higher glucose measurements in Dcn−/− mice in a state of satiety, glucose tolerance was equivalent between wild-type and Dcn−/− mice (Fig. 2B), suggesting intact glucose clearance mechanisms. Moreover, fed and fasted plasma insulin levels were nearly identical between wild-type and Dcn−/− mice, and both genotypes responded appropriately to fasting by lowering circulating insulin levels (Fig. 2C).

Looking at other global and cardiac metabolic markers, we again found no major changes between wild-type and Dcn−/− mice. Specifically, quantification of cardiac glycogen was similar (Fig. 2D), as were measurement of circulating and cardiac free fatty acids, both in the fed and fasted state (Fig. 2E and 2F). Hence, wild-type and Dcn−/− mice demonstrate comparable global metabolic parameters, including glucose clearance and insulin sensitivity with no major perturbations in glycogen turnover or fatty acid metabolism.

High-throughput metabolomics analysis reveals abnormal cardiac glucose utilization in Dcn−/− mice

Next, we considered metabolic differences in wild-type and Dcn−/− cardiac tissue as they related to nutrient catabolism. To this end, we performed an unbiased, high-throughput mass spectrometry-based metabolomics study (see also supporting dataset: Iozzo Metabolomics). Intriguingly, many differences were noted in pathways involving glucose utilization, especially following fasting. We detected a common pattern, whereby fasting resulted in increased levels of certain metabolites in
wild-type samples, but this increase was either attenuated or not evident in Dcn<sup>−/−</sup> hearts. For example, glucose and glucose-6-phosphate were significantly higher in fasted versus fed wild-type hearts (Fig. 3A and 3B). However, this same amount of nutrient deprivation resulted in no significant increase in fasted glucose and a much lower increase in fasted levels of glucose-6-phosphate in Dcn<sup>−/−</sup> cardiac tissue (Fig. 3A and 3B). Indeed, the fasted levels of these metabolites were significantly lower than the amounts measured in wild-type fasted samples (Fig. 3A and 3B).

Since glucose and glucose-6-phosphate are shuttled through several different pathways, we next examined glycolytic intermediates. We found that 1,6-fructose-bisphosphate followed the aforementioned paradigm, with a blunted response in the fasted state in Dcn<sup>−/−</sup> hearts (Fig. 3C). In contrast, the late glycolytic intermediates, 3-phosphoglycerate and its isomer 2-phosphoglycerate, trended toward being higher than the wild-type levels, particularly in the fasted state (Fig. 3D and 3E). Analysis of the citric acid cycle intermediates aconitate and succinate revealed minimal changes with fasting in wild-type hearts (Fig. 3F and 3G). In contrast, fasting resulted in decreased levels in Dcn<sup>−/−</sup> cardiac samples (Fig. 3F and 3G). Fasted wild-type hearts also exhibited increased fumarate, whereas fasting did not alter Dcn<sup>−/−</sup> fumarate levels, though the differences between genotypes in the fasted state were not significant (Fig. 3H).

Like glucose, the pentose phosphate pathway intermediates, 6-phosphogluconate and sedoheptulose-7-phosphate were increased in fasted wild-type mice, whereas no significant changes were found with fasting in Dcn<sup>−/−</sup> hearts (Fig. 3I and 3J). Additionally, the UDP-sugars, UDP-glucose and UDP-galactose, were significantly decreased with fasting in wild-type hearts, but maintained or less significantly decreased with fasting in Dcn<sup>−/−</sup> mice (Fig. 3K and 3L).

Given these differences and given that the heart typically uses fatty acid oxidation under starvation conditions, we focused on glycolytic genes to investigate the possibility that the Dcn<sup>−/−</sup> hearts primarily used glycolysis rather than lipid metabolism during this stressor (Fig. S3A, S3B, S3C, and S3D). However, while we found differential expression between genotypes of Pgam2 and Eno1 following fasting for 25 h (Fig. S3C and S3D), we did not find any significant alterations in protein expression at this time point (Fig. S3E, S3F, S3G, and S3H). There did appear to be lower levels of Eno1 protein expression in Dcn<sup>−/−</sup> hearts that had undergone 48 h of fasting (Fig. S3F and S3H). However, given that metabolomics data were obtained at the 25 h time point, we did not follow this finding. Thus, we considered an alternate glucose utilization pathway occurring at 25 h in order to explain these metabolic differences.

**Dcn<sup>−/−</sup> hearts augment flux through the hexosamine biosynthetic pathway following fasting resulting in increased cardiac O-GlcNAcylation**

Though only 2-5% of glucose enters the HBP, we scrutinized several of its intermediates, as it is a known nutrient, and particularly glucose, sensing pathway. We identified the consistent increase in many metabolites with fasting in wild-type hearts (fructose, glutamine, glucoseamine-6-phosphate), which was attenuated in fasted Dcn<sup>−/−</sup> samples (Fig. 4A, 4B, and 4C). Notably, in addition to less pronounced increases with fasting, many of these Dcn<sup>−/−</sup> fasted measurements were significantly lower than the levels in fasted wild-type tissue (Fig. 4A, 4B, and 4C). Of interest, fasting decreased Dcn<sup>−/−</sup> N-acetylglucosamine-6-phosphate, whereas its epimer, N-acetylglucosamine-1-phosphate, showed only modest differences (Fig. 4D and 4E). Perhaps most noteworthy, uridine diphosphate (UDP) levels were barely detectable in fasted wild-type hearts (Fig. 4F). However, though a slight decrease was apparent in fasted Dcn<sup>−/−</sup> cardiac tissue, these levels were significantly higher than those seen in the wild-type samples (Fig. 4F).

We must emphasize that this analysis was executed after 25 h of fasting, as the differences in cardiac autophagy levels between wild-type and Dcn<sup>−/−</sup> mice were unmistakable even with this standard period of nutrient deprivation. Thus, since these measurements were taken at one static time point, these metabolic differences may be due to either increased or decreased flux along these pathways. To gain insight into HBP flux, we analyzed the expression of glucose fructose-6-phosphate amidotransferase (Gfat), the rate-limiting enzyme of this pathway. We specifically studied Gfat2, as this isoform is most highly expressed in the heart. Remarkably, 25 h of fasting
significantly suppressed the expression of this protein in wild-type hearts (Fig. 4G and 4H). On the contrary, expression of Gfat2 remained constant with fasting in Dcn−/− cardiac tissue (Fig. 4G and 4H). Following these findings, we assessed total O-GlcNAcylation and found that fasting did not significantly alter this post-translational modification in wild-type hearts, though there was a slight trend towards decreased levels. In contrast, fasting caused a nearly significant increase in O-GlcNAcylation in Dcn−/− cardiac tissue vis-à-vis fed equivalents (Fig. 4I and 4J). In fact, fasted cardiac O-GlcNAcylation was significantly higher in Dcn−/− mice when compared with fasted wild-type counterparts (Fig. 4J). We conclude that Dcn−/− mice utilize the HBP in cardiac tissue to a greater extent than wild-type mice during fasting resulting in amplified global cardiac protein O-GlcNAcylation.

**Dcn−/− mice preserve ejection fraction with fasting, which can be reversed by systemic delivery of recombinant decorin**

With regard to cardiac function, autophagy holds dual roles as both a protective and pathological mechanism (28). Additionally, proper metabolism is particularly important for maintaining cardiac homeostasis. As Dcn−/− mice exhibit impaired cardiac autophagy and aberrant glucose metabolism, we posited that lack of decorin would cause anomalous cardiac function. Structurally, we found no inherent differences between wild-type and Dcn−/− hearts in terms of diastolic left ventricular internal diameter (LVID), left ventricular posterior wall thickness (LVPW), or interventricular septal diameter (IVS) (Fig. 5A, 5B, and 5C). Thus, any functional differences arising between the two genotypes would not be due to structural disparities, but rather due to purely functional alterations. In addition, no changes were noted between genotypes in the following hemodynamic properties: systolic pressure (SP), left ventricular end diastolic pressure (LVEDP), and heart rate (HR) (Fig. 5D, 5E, and 5F).

Via M-mode echocardiography (Fig. 6A, 6B, 6C, 6D, and 6E), we calculated the ejection fraction for both fed and fasted wild-type and Dcn−/− mice (see also Supplemental Videos 1-5). In agreement with published data (29,30), we found that fasting significantly reduced ejection fraction in wild-type mice (Fig. 6F). Surprisingly, ejection fraction was maintained with fasting in Dcn−/− mice (Fig. 6F).

Most importantly, treatment with decorin protein core reverted the fasted Dcn−/− ejection fraction to levels similar to those found in the fasted wild-type mice (Fig. 6F). Hence, we conclude that decorin is a critical nutrient sensor that is imperative for the maintenance of normal cardiac function during low-energy states where its absence results in insensitivity to pro-autophagic cues, altered downstream O-GlcNAcylation, and inability to properly reduce cardiac output as a means of energy preservation (Fig. 6G).

**DISCUSSION**

The role of the extracellular matrix as a key regulator of intracellular processes and organism function is becoming increasingly visible (31-40). Decorin is a keystone of this paradigm, where it is implicated in a myriad of signaling pathways to maintain a healthy environment as well as prevent pathology associated with many different diseases, particularly cancer. Our current study provides further evidence that decorin is a significant regulator of critical cellular pathways via receptor-mediated signaling where we have defined a new role for it as a nutrient sensor that modulates cardiac autophagy and metabolism. Taken together, these functions position decorin as a fundamental signaling effector to control cardiac output, especially under nutrient-deficient conditions.

Our recent findings that Dcn−/− mice exhibit reduced cardiac autophagy even with prolonged nutrient deprivation and the ability of exogenous decorin to provoke an autophagic response in these same mice provide a new model for outside-in signaling. Specifically, the fact that decorin only rescues autophagic levels in vivo following fasting suggests that it is not so much the mere presence of decorin, but rather its localization and/or binding partners during nutrient deprivation that promote autophagy. Our investigation of Igf1r signaling supports this idea in that in both the fed and fasted states of Dcn−/− hearts, there is minimal phosphorylation of this receptor, signifying insensitivity to extracellular cues in the absence of decorin. As decorin binds and signals through Igf1r via its protein core, it is likely that this receptor-ligand interaction is responsible for these findings. Alternatively, as decorin binds several growth factors, including Igf-1 (1,19) this deviant signaling could also be due to impaired presentation of the growth factor to its receptor. However, given that
recombinant decorin core was able to restore normal autophagy in response to fasting, it is more likely that the former rather than the latter is at play. Further study must be undertaken in this avenue to parse out the exact role that decorin plays in these processes with a focused emphasis on which binding partners are members of this intricate assembly.

Given the differential phosphorylation of cardiac Igf1 and in the absence of decorin, we were somewhat surprised to find few alterations in common metabolism markers between wild-type and Dcn−/− mice. While the Dcn−/− mice display elevated fed blood glucose levels, they are not glucose intolerant or insulin resistant, suggesting they are not overtly diabetic or even pre-diabetic. Intriguingly, anecdotal observations from our lab note that some, but not all, Dcn−/− mice demonstrate increased abdominal adiposity when compared with wild-type mice, especially as they age. Interestingly, proteomic analysis of obese patients illustrates an accumulation of decorin in adipose tissue (41). However, these studies were purely observational and no real function for increased decorin expression in adipose tissue has been elucidated as of yet. Therefore, we believe that decorin is important for global metabolic homeostasis, although the exact mechanism is still to be determined.

Despite only subtle global metabolic differences, we were encouraged to find conspicuous cardiac metabolic disparities vis-a-vis wild-type mice, particularly prominent in the HBP. Importantly, an independent metabolomics study similarly reported altered cardiac metabolism in Dcn−/− mice (42), though in the context of atrial fibrillation and not starvation. These metabolic aberrations may help explain the Dcn−/− autophagic defect, as recent evidence supports the hypothesis that increased O-GlcNAcylation inhibits autophagy. In a diabetic model, pharmacologically increasing O-GlcNAcylation blunts autophagic signaling specifically in cardiac tissue (13). Other studies provide a role for O-GlcNAcylation as a means to prevent autophagosome maturation by interfering with SNARE proteins (43). In addition, Torin 1, a potent pro-autophagic compound, inhibits O-GlcNAc transferase, the enzyme that catalyzes O-GlcNAcylation, while simultaneously inducing the antithetic O-GlcNAcase, which removes these post-translational modifications (44). Finally, loss of expression of Gfat, the HBP rate-limiting enzyme, enhances autophagy as measured by increases in LC3-positive puncta (43). Moreover, acute increases in O-GlcNAcylation can act as a stress response. Thus, the higher baseline levels of glucose in the fed state and increased O-GlcNAcylation seen in fasted Dcn−/− cardiac tissue indicate that this altered sugar usage could be an unorthodox mechanism in response to nutrient-related stress, which may directly inhibit an autophagic response. Perhaps even more interestingly, our earlier work shows that decorin is upregulated in the heart during periods of fasting (11). Hence, it is possible that, during fasting, the wild-type heart moves toward a biosynthetic state resulting in the diversion of UDP-GlcNAc into proteoglycan synthesis whereas in the absence of decorin, the cardiac ECM improperly shunts this moiety toward global O-GlcNAcylation.

Our findings regarding cardiac functional differences were quite remarkable. Just as we found, other studies report that fasting reduces ejection fraction (29,30). Though autophagy may be the main mechanism to recycle nutrients to sustain cardiac homeostasis in wild-type hearts, the reduction in ejection fraction may be an additional failsafe to further support cardiomyocyte survival when food availability is low. In contrast, the ability for Dcn−/− mice to maintain cardiac output with fasting aligns with our previous observation that these mice do not detect differences in nutrient status. Interestingly, data from a hemorrhagic model study show that glucosamine infusion increases cardiac O-GlcNAcylation and cardiac output (45). Therefore, the increased O-GlcNAcylation during fasting may be the cause of sustained ejection fraction in the Dcn−/− mice. Of note, while this preservation of cardiac function with fasting appears at first glance to be advantageous, it may also be problematic as continual normal cardiac function in the context of reduced nutrient supply may lead to adverse events, such as myocardial infarction, especially if nutrient deprivation is combined with another stressor, such as extreme exercise or sepsis.

Additionally, we must comment that O-GlcNAcylation enhances the expression of hyaluronan synthase 2 (HAS2), a critical mediator of hyaluronan synthesis (46,47). Though our study did not address this consequence of increased O-GlcNAcylation, it is possible that there exists
aberrant hyaluronan expression and signaling in these Dcn−/− hearts, particularly during fasting. Additionally, especially under periods of prolonged nutrient deprivation, there may be a complex interplay between the adenosine monophosphate kinase (AMPK)-signaling axis and HAS2 regulation, as AMPK activation has been shown to reduce HAS2 activity (48). Further investigation is warranted to identify a connection among the autophagic defect including AMPK-signaling, the preserved ejection fraction and the hyaluronan-signaling pathway in the absence of decorin.

Taken together, these new findings illuminate decorin as a fundamental extracellular signaling molecule that simultaneously regulates cardiac autophagy, metabolism, and function during nutrient deprivation. Given this information, decorin may prove to be important as both a prognostic and diagnostic marker for heart disease and could also be an effective therapeutic option to regulate cardiac metabolism in the setting of obesity or diabetes. Furthermore, we believe that future investigation will yield many more extracellular proteoglycans and other associated matrix members as key players in this elaborate network. Thus, we provide an additional meaningful function to decorin’s already extensive repertoire and further underscore the importance of the extracellular matrix for normal cell signaling.

**EXPERIMENTAL PROCEDURES**

**Chemicals and antibodies**

Antibodies were purchased as follows: Actb (Abcam, ab8227), Beclin 1 (Cell Signaling 3738), Eno1, (Abcam, ab49343), Gapdh (Cell Signaling, 14C10), Gfat2 (Abcam, ab155926), total Igf1r (Santa Cruz, sc-713), P-Igf1r Tyr1135/36 (Cell Signaling, 3024), LC3 (Sigma, L7543), O-GlcNAc CTD.110.6 (Cell Signaling, 9875) and Phosphoglycerate mutase 2 (Abcam, ab187147).

All antibodies were used at a 1:1000 dilution for Western blot and 1:500 for immunofluorescence except Actb and Gapdh, which were used at 1:10,000. Decorin was purified as previously described (49). Goat anti-rabbit and anti-mouse secondary antibodies conjugated with HRP (12-348, 12-349) were purchased from Millipore, Inc. and used at 1:4000 dilution. Torin 1(42-471-0) was purchased from Tocris.

**Animal experiments**

C57BL/6 mice were purchased from Jackson Laboratories. Global Dcn−/− mice were generated as previously described (50). Animal experiments were performed as per the Guide for Care and Use of Laboratory Animals and the Institutional Animal Care and Use Committee of Thomas Jefferson University. Mice were of both male and female sex ranging from 2-4 months of age. Fasting experiments involved withholding food for 25 or 48 h, but water was allowed *ad libitum*. Equal numbers of male and female mice were used for each experiment. After animals were euthanized, organs were removed and immediately snap-frozen in liquid N2. For rescue experiments, mice were injected intraperitoneally with 10 mg/kg of purified human decorin protein core every other day for one week before fasting and sacrifice. For in vivo flux experiments, mice were fasted for 25 h and 6 h before sacrifice were injected intraperitoneally with 100 mg/kg chloroquine. Torin 1 was administered via intraperitoneal injection at a concentration of 5 mg/kg.

**Echocardiography**

Cardiac function was assessed via echocardiography, which was performed with the VisualSonics VeVo 2100 imaging system in animals anesthetized with 1.5% vol/vol isoflurane. The internal diameter of the left ventricle was measured in the short-axis view from M-mode recordings in end diastole and end systole. Hemodynamic measurements were obtained via carotid artery catheterization.

**Metabolomics analysis**

Cardiac tissue from wild-type and Dcn−/− mice that had been either fed *ad libitum* or subjected to 25 h fasting were analyzed by Metabolon, Inc. (http://www.metabolon.com/technology.aspx) using a pre-determined dataset of 554 compounds. Cardiac metabolites were measured with a high-resolution accurate mass (HRAM) platform of ultrahigh performance liquid chromatography/mass spectrometry (LC-MS) and gas chromatography/mass spectrometry (GC-MS). All analyses included acquisition of raw data, peak identification, and comparison with several quality control samples. The complete dataset can be found at IozzoLab.com under the Metabolomics tab.

**Measurement of metabolic parameters**
Blood glucose was measured using a standard blood glucometer. Insulin levels were assessed using an ELISA specific for mouse insulin (Crystal Chem, 90080). Glycogen and free fatty acid levels were measured using colorimetric assays as per the manufacturer’s directions (Sigma, MAK016 and MAK044). For glucose tolerance tests, mice were fasted for 4 h followed by oral gavage of 2g/kg 25% glucose solution. Blood glucose was measured via glucometer at 0, 15, 30, 60, 90, and 120 min.

**Western blot and immunofluorescence analysis**
Tissue samples were lysed in T-Per Reagent with EDTA and protease inhibitor (Life Technologies, 78510). Samples were separated by SDS-PAGE and transferred to nitrocellulose where specific antibodies were used to visualize the proteins. Immunofluorescence was performed as previously described (51,52). In brief, cardiac sections were fixed with paraformaldehyde and stained with an LC3-specific antibody. Nuclei were visualized with DAPI. Quantification of LC3 puncta was performed using a macro specific for ImageJ software that was designed by Ruben Dagda (University of Nevada School of Medicine) (53,54).

**Quantitative real-time PCR analysis**
One mm$^3$ of tissue was lysed in 750 µl of TRIzol® reagent (Life Technologies, 15596-026). RNA was isolated using a standard RNA isolation kit (Zymo Research, R2052). 1 µg of RNA was annealed with oligo(dT) primers (Life Technologies, 18418-012) and cDNA was synthesized using SuperScript Reverse Transcriptase II (Life Technologies, 18064-022) according to the manufacturer’s directions. The target genes (primer sequences can be found in Supplemental Table 1) and housekeeping gene (Actb) were amplified in independent reactions using the Brilliant SYBR Green Master Mix II (Agilent Technologies, 600828). Samples were run in duplicate on a LightCycler480-II (Roche Applied Science) and the cycle number ($C_t$) was obtained for each reaction. Fold-change determinations were made utilizing the comparative $C_t$ method for gene expression analysis.

**Quantification and statistical analysis**

**ABBREVIATIONS**
AMPK, adenosine monophosphate kinase

Experiments were repeated three or more times and all data are expressed as means ± SEM. Paired and unpaired two-tailed Student’s $t$-tests were used to analyze significance using the SigmaStat program. $p<0.05$ was considered statistically significant. For the metabolomics analysis, following log transformation and imputation of missing values, if any, with the minimum observed value for each compound, ANOVA contrasts were used to identify biochemicals that differed significantly between experimental groups. A summary of the numbers of biochemicals that achieved statistical significance ($p\leq0.05$), as well as those approaching significance ($0.05<p<0.10$) were noted (see Iozzo Metabolomics dataset). Analysis by two-way ANOVA identified biochemicals exhibiting significant interaction and main effects for experimental parameters of genotype and feed status.

**ACKNOWLEDGEMENTS**
Thank you to R.T. Owens for providing the recombinant decorin, and Nadan Wang of the Small Animal Physiology Core of Thomas Jefferson University for his technical expertise and guidance of the cardiac functional studies.

This work was supported in part by National Institutes of Health Grants RO1 CA39481, RO1 CA47282, and RO1 CA164462 (to R. V. I.). M. A. Gubbiotti was supported in part by NIH training grant T32 AA07463.

**DECLARATION OF INTERESTS**
The authors declare that they have no conflicts of interest with the contents of this article.

**AUTHOR CONTRIBUTIONS**
Conceptualization, R.V.I., M.A.G., and J.H.; Methodology, R.V.I., M.A.G., E.S., J.H., and U.R.; Investigation, M.A.G., R.V.I., and E.S.; Formal Analysis, M.A.G., R.V.I., J.H., and U.R.; Writing – Original Draft, M.A.G. and R.V.I.; Writing – Reviewing & Editing, M.A.G., J.H., E.S., U.R., and R.V.I.; Funding Acquisition, R.V.I.; Supervision, R.V.I. and J.H.
Decorin senses nutrient status for proper cardiac function

CQ, chloroquine
Dcn, decorin
Eno1, enolase 1
Gfat, glutamine fructose amidotransferase
HAS2, hyaluronan synthase 2
HBP, hexosamine biosynthetic pathway
Hk2, hexokinase II
HR, heart rate
Igf1r, insulin growth factor receptor 1
Igf-1, insulin-like growth factor 1
IVS, interventricular septal thickness
LC3, microtubule-associated light chain protein 3
LVEDP, left ventricular end diastolic pressure
LVID, left ventricular inner diameter
LVPW, left ventricular posterior wall thickness
Map1lc3a, microtubule-associated light chain protein 3 gene
mTOR, mammalian target of rapamycin
O-GlcNAcylation, O-β-N-Acetylglucosylation
Pfk, phosphofructokinase muscle isoform
Pgam2, phosphoglycerate mutase 2
RTK, receptor tyrosine kinase
SLRP, small leucine-rich proteoglycan
SP, systolic pressure
Tyr, tyrosine
UDP, uridine disphosphate
Decorin senses nutrient status for proper cardiac function

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Decorin senses nutrient status for proper cardiac function

Figure 1. Exogenous decorin treatment, but not prolonged nutrient deprivation, restores fasting-induced autophagy in Dcn−/− hearts. A, representative Western blot of LC3 protein levels in wild-type and Dcn−/− hearts following 48 h of fasting. B, quantification of (A) via densitometric analysis. C-F, representative sections of fed and fasted wild-type and Dcn−/− cardiac tissue demonstrating LC3-positive puncta (red). Nuclei are stained with DAPI (blue). Arrows denote the puncta. Scale bar ~10 μm. G, quantification of LC3-positive puncta seen in C-F. H, fed and fasted Map1lc3a expression levels in wild-type and decorin-treated Dcn−/− hearts. I, western blot for cardiac LC3 protein levels in fed and fasted wild-type and decorin-treated Dcn−/− mice. J, quantification of (I). K, phosphorylated (Tyr1135/36) and total Igf1r expression in wild-type and Dcn−/− hearts in fed and fasted states. L, quantification of P-Igf1r/total Igf1r in (K). Data represent means ±SEM. p-values calculated using Student’s t-test.
Figure 2. *Dcn*<sup>−/−</sup> mice exhibit global metabolism comparable to wild-type mice. A, blood glucose measurements of fed, 25-h fasted, and 48-h fasted wild-type and *Dcn*<sup>−/−</sup> mice. B, glucose tolerance curves of wild-type and *Dcn*<sup>−/−</sup> mice at designated time points. C, fed and fasted wild-type and *Dcn*<sup>−/−</sup> plasma insulin levels measured via ELISA. D, quantification of fed and fasted cardiac glycogen levels. E and F, fed and fasted free fatty acid levels in wild-type and *Dcn*<sup>−/−</sup> mice in plasma (E) and cardiac tissue (F). Data points represent individual animals. In (B), data represent means ±SEM. *p*-values calculated using Student’s *t*-test.
Decorin senses nutrient status for proper cardiac function

Figure 3. High-throughput metabolomics analysis provides evidence that Dcn<sup>-/-</sup> mice demonstrate anomalous cardiac glucose utilization. Mass spectrometry analysis of fed and 25-h fasted wild-type and Dcn<sup>-/-</sup> cardiac tissue including: A,B, glucose and glucose-6-phosphate. C-E, glycolytic intermediates F-H, citric acid cycle byproducts. I,J, pentose phosphate pathway metabolites. K,L, UDP-sugars. Data points represent individual animals. p-values calculated via ANOVA contrasts.
Figure 4. Dcn<sup>−/−</sup> mice augment flux through the hexosamine biosynthetic pathway during fasting resulting in increased cardiac O-GlcNAcylation. A–F, mass spectrometry analysis of hexosamine biosynthetic pathway intermediates in the heart. G, representative Western blot of Gfat2 in fed and 25-h fasted wild-type and Dcn<sup>−/−</sup> cardiac tissue. H, quantification of (G). I, analysis of global cardiac O-GlcNAcylation following 25-h fasting in wild-type and Dcn<sup>−/−</sup> mice. J, quantification of (I). Data represent means ±SEM. *p*-values calculated using either ANOVA contrasts (4A–F) or Student’s *t*-test (4H and 4J).
Figure 5. There are no apparent structural disparities between wild-type and Dcn<sup>−/−</sup> hearts. Echocardiography analysis of A, left ventricular inner diameter (LVIDd), B, left ventricular posterior wall thickness (LVPWd), and C, interventricular septal diameter (IVSd) in diastole. Hemodynamic analysis of: D, systolic pressure (SP), E, left ventricular end diastolic pressure (LVEDP), F, heart rate (HR). Data represent means ±SEM. p-values calculated using Student’s t-test.
Figure 6. *Dcn*<sup>−/−</sup> mice preserve ejection fraction following fasting, which can be reverted to wild-type levels upon decorin treatment. *A-E*, representative echocardiograms in fed and fasted wild-type and *Dcn*<sup>−/−</sup> hearts. Blue lines represent LVID in diastole and red lines represent LVID in systole. Videos can be found in the Supplemental Information (SVideo1-5). *F*, quantification of calculated ejection fraction as seen in (A-E). *G*, schematic demonstrating the finding that decorin is necessary to invoke autophagy and reduce ejection fraction during starvation. Data represent mean ±SEM. *p*-values calculated using Student’s *t*-test.
Metabolic reprogramming of murine cardiomyocytes during autophagy requires the extracellular nutrient sensor, decorin
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J. Biol. Chem. published online July 26, 2018

Access the most updated version of this article at doi: 10.1074/jbc.RA118.004563

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