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Adropin treatment restores cardiac glucose oxidation in pre-diabetic obese mice

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Abstract

Exposure to a high fat (HF) diet promotes increased fatty acid uptake, fatty acid oxidation and lipid accumulation in the heart. These maladaptive changes impact cellular energy metabolism and may promote the development of cardiac dysfunction. Attempts to increase cardiac glucose utilization have been proposed as a way to reverse cardiomyopathy in obese and diabetic individuals. Adropin is a nutrient-regulated metabolic hormone shown to promote glucose oxidation over fatty acid oxidation in skeletal muscle homogenates *in vitro*. The focus of the current study was to investigate whether adropin can regulate substrate metabolism in the heart following prolonged exposure to a HF diet *in vivo*. Mice on a long-term HF diet received serial intraperitoneal injections of vehicle or adropin over three days. Cardiac glucose oxidation was significantly reduced in HF animals, which was rescued by acute adropin treatment. Significant decreases in cardiac pyruvate dehydrogenase activity were observed in HF animals, which were also reversed by adropin treatment. In contrast to previous studies, this change was unrelated to *Pdk4* expression, which remained elevated in both vehicle- and adropin-treated HF mice. Instead, we show that adropin modulated the expression of the mitochondrial acetyltransferase enzyme

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8. CONFLICTS OF INTEREST

None.

GCN5L1, which altered the acetylation status and activity of fuel metabolism enzymes to favor glucose utilization. Our findings indicate that adropin exposure leads to increased cardiac glucose oxidation under HF conditions, and may provide a future therapeutic avenue in the treatment of diabetic cardiomyopathy.

GRAPHICAL ABSTRACT

Keywords

Adropin; Mitochondria; Fatty Acid Oxidation; Glucose Oxidation; Metabolism; Acetylation

2. INTRODUCTION

Cardiac mitochondria supply around 90% of the energy required for contractile function via oxidative phosphorylation. Under normal conditions, most of this energy is generated from the oxidation of fatty acids, with the remainder coming from alternative sources such as glucose, lactate and ketones [1]. While hearts have a clear preference for fatty acids under normal conditions, they maintain a level of fuel substrate flexibility to continue contractile function under stress. This flexibility is lost in disease states such as diabetic cardiomyopathy, where increased plasma fatty acid levels and decreased glucose uptake can lead to an over-reliance on fatty acid oxidation for ATP generation. This results in decreased cardiac energy efficiency, which may exacerbate the bioenergetic deficits that characterize metabolic disease states [2].

To repair the metabolic dysfunction found in cardiovascular diseases, one pathway of research has focused on the pharmacological inhibition of cardiac fatty acid oxidation to promote glucose utilization (a more efficient fuel in terms of energy produced per mole of oxygen used [2]). These studies resulted in the development of several drugs (e.g. etomoxir, perhexiline, trimetazidine) that showed great therapeutic potential, but have had limited clinical success due to off-target metabolic effects [3]. Alternative strategies to promote a switch from fatty acid to glucose utilization in the diabetic heart have therefore been sought.

Adropin is a liver- and brain-derived peptide shown to reduce insulin resistance in mice subject to diet-induced obesity [4]. Recent work has demonstrated that adropin can promote the use of glucose as an oxidation substrate in skeletal muscle homogenates isolated from diabetic animals, driven by changes in the expression of fatty acid oxidation and glucose utilization genes [5,6]. However, it remained unclear from these studies if adropin would produce these potentially beneficial effects *in vivo*. As such, in this study we sought to investigate if adropin could perform a similar function and restore glucose oxidation in the hearts of pre-diabetic obese mice.

3. MATERIALS AND METHODS

Detailed methods are available in the attached supplemental material.

4. RESULTS

4.1 Adropin treatment restores cardiac glucose oxidation in obese mice

Mice placed on a long-term, high fat (HF) diet develop changes in cardiac metabolism, which results in increased fatty acid uptake, cardiomyocyte lipid accumulation, and contractile dysfunction [7]. We sought to determine whether glucose oxidation is modulated by adropin treatment in this murine model of cardiac metabolic dysfunction. Age- and weight-matched mice on a 20-week HF diet received five serial intraperitoneal injections of vehicle (saline) or adropin (450 nmol/kg) over three days, as previously described [6]. Relative rates of cardiac-specific glucose versus fatty acid oxidation (V_{PDH}/V_{TCA}) were measured in response to a physiological stimulus (hyperinsulinemia) using a universally-labeled U- ^{13}C glucose infusion to match plasma glucose levels between groups (Figure 1A). Mean body weights were significantly different between both HF groups (vehicle or adropin) and chow fed, vehicle-treated controls, before and after catheterization surgery (Figure 1B-C). Both HF groups displayed significantly increased plasma insulin levels, and mildly elevated (but non-significant) plasma glucose levels, relative to chow fed mice following a 6 hour fast (Figure 1D-E). The glucose infusion rate to maintain euglycemia during the clamp was significantly reduced in both HF diet groups, indicating systemic metabolic dysfunction (Figure 1F). Plasma insulin levels were matched during the infusion between vehicle- and adropin-treated HF groups (Figure 1G); however, vehicle-treated chow mice displayed modestly reduced plasma insulin levels despite identical infusion rates, suggesting impaired insulin clearance and hepatic insulin resistance in the HF-fed groups [8]. Similarly, plasma glucose levels were matched between HF groups, whereas plasma glucose levels were modestly less in chow mice compared with HF groups (Figure 1H).

Under hyperinsulinemic clamp conditions, 67% of carbon flux through the TCA cycle was attributed to glucose oxidation in vehicle-treated chow mice, whereas only 30% of TCA cycle flux was attributed to glucose oxidation in the vehicle-treated HF mice (Figure 1I). Adropin treatment significantly restored glucose flux through pyruvate dehydrogenase (PDH) to 54% of carbon flux through the TCA cycle, thereby rescuing the defect seen in vehicle-treated HF mice (Figure 1I). This indicates that adropin treatment has the capacity to regulate cardiac energy metabolism, and can promote cardiac glucose oxidation under conditions that typically restrict its use.

4.2 Adropin promotes cardiac glucose oxidation independently of PDK4 via metabolic enzyme deacetylation

In agreement with our *in vivo* studies, *in vitro* assays using cardiac tissue showed that there was a significant decrease in PDH activity in HF treated animals relative to chow-fed controls, which was reversed by adropin treatment (Figure 2A). We recently showed that in cardiac-derived H9c2 cells, adropin exposure reduced the expression of the PDH negative regulatory kinase, *Pdk4* [9]. We examined gene expression in each treatment cohort, and

found that in contrast to previous *in vitro* findings, there remained a significantly elevated expression of *Pdk4* in both HF groups relative to chow-fed controls (Figure 2B). Furthermore, we found that PDH phosphorylation (S293) remained elevated in both HF groups (Figure 2C), indicating that changes in glucose utilization are not dependent on this regulatory pathway

Lysine acetylation, a reversible PTM that uses nutrient-derived acetyl-CoA as a modifying co-factor, has been documented to control the enzymatic activity of mitochondrial fuel metabolism enzymes in the heart, including LCAD and PDH [10,11,12]. We therefore sought to determine if the regulatory effect of adropin on cardiac glucose oxidation was mediated by changes in metabolic enzyme lysine acetylation. We first measured the abundance of GCN5L1, an acetyltransferase previously demonstrated to regulate lysine acetylation in the heart [10,13]. We found that GCN5L1 was significantly elevated in HF cardiac tissues relative to chow-fed mice, which was reversed by adropin treatment (Figure 2D).

Previous research in the heart has shown that increased lysine acetylation promotes the activity of fatty acid oxidation enzymes such as LCAD, and inhibits the activity of PDH [10,11,12]. As such, we next tested whether the changes in GCN5L1 expression would lead to alterations in the acetylation of specific metabolic enzymes. Relative to chow-fed animals, there was a significant increase in PDH acetylation in the HF group (Figure 2E). In addition, there was a significant increase in the acetylation status of the fatty acid oxidation enzyme Hydroxyacyl-CoA Dehydrogenase Subunit Alpha (HADHA), with a trend towards increased acetylation in LCAD (Supplemental Figure 1A-B). Treatment with adropin reduced PDH acetylation close to levels seen in chow-fed animals (Figure 2E), and greatly reduced the acetylation of both fatty acid oxidation enzymes relative to vehicle-treated HF animals (Supplemental Figure 1A-B). We next examined whether acetylation status was related to enzyme activity in these animal cohorts, and found that there was a significant negative correlation between PDH activity and acetylation abundance, and a significant positive correlation between LCAD activity and acetylation abundance (Figure 2F, Supplemental Figure 1C). Finally, we examined whether increased GCN5L1 abundance could affect the ability of adropin to upregulate PDH activity acutely. Adropin treatment led to a significant increase (~60%) in PDH activity in H9c2 cells transfected with an empty vector (Figure 2G-I). In contrast, cells over-expressing GCN5L1 did not display any significant changes in PDH activity following adropin treatment (Figure 2G-I), mirroring the effects of HF diet-related increases in GCN5L1 abundance. Based on our findings, we conclude that adropin may drive glucose utilization in the hearts of obese mice by altering the acetylation status of mitochondrial enzymes to favor mitochondrial glucose oxidation activity.

5. DISCUSSION

In this study, we show for the first time that adropin can regulate fuel substrate utilization *in vivo*. Treatment of obese, pre-diabetic mice with adropin restored relative cardiac glucose oxidation rates to those seen in lean animals, significantly reversing the metabolic dysfunction observed in vehicle-treated high fat diet animals. Unlike previous studies in skeletal muscle, the ability of adropin to promote glucose oxidation was independent of the

inhibitory effect of PDK4 on pyruvate dehydrogenase activity. Instead, we provide evidence that adropin treatment regulates the acetylation status of mitochondrial fuel utilization enzymes, in a manner that promotes glucose utilization at the expense of fatty acid oxidation.

Previous studies have shown that adropin regulates mitochondrial glucose utilization via PDK4-dependent changes in PDH phosphorylation in skeletal muscle homogenates *in vitro* [5,6]. While altered *Pdk4* gene expression is seen in response to adropin exposure in H9c2 cells *in vitro* [9], there is no obvious regulatory role for PDK4-mediated phosphorylation in response to this peptide in the heart *in vivo* (Figure 2). Instead, we posit a mechanism whereby adropin exposure alters the activity of key mitochondrial fuel metabolism enzymes by reducing their acetylation status. Our group and others have shown that hyperacetylation of LCAD and HADHA in the heart increases their enzymatic activity, while concomitantly reducing the activity of PDH [10,11,12]. This is in direct contrast to the liver, where hyperacetylation of LCAD reduces its enzymatic activity and leads to the development of metabolic syndrome [14]. In the current study, we show that adropin exposure in the heart reduces the abundance of the mitochondrial acetyltransferase-related enzyme GCN5L1, resulting in opposing effects on enzyme activity for LCAD and PDH (Figure 2). The novel links between mitochondrial acetylation status and adropin abundance remain to be fully explored, and suggest a possible new mechanistic link between nutritional status and mitochondrial metabolic activity.

Although there is some disagreement about whether insulin resistance is adaptive or maladaptive for the failing heart [15], loss of insulin stimulated glucose oxidation is a major metabolic change that occurs in diabetic cardiomyopathy. In our HF-induced pre-diabetic mouse model, we show that adropin can restore glucose oxidation in otherwise glucose intolerant mice (Figure 1). Our acute studies were specifically designed to explore the remodeling of fuel metabolism in the heart, and as expected this short-term treatment over three days did not lead to significant changes in cardiac contractile function (Supplemental Figure 2). Despite this, our acute adropin regimen led to a small reduction in mean left ventricular wall thickness and a minor improvement in E/A wave ratio (a marker of diastolic dysfunction) relative to vehicle-treated HF mice (Supplemental Figure 2). Future studies on the chronic effect of adropin on cardiac functional parameters are planned, and will address whether long-term glucose oxidation upregulation in the diabetic heart leads to adaptive improvements in cardiac function.

The current study provides robust evidence that adropin modulates cardiac energy metabolism in obese, pre-diabetic mice. These pre-clinical data suggest that targeting the adropin signaling pathway may be an effective approach to reinstate cardiac glucose utilization in obese individuals, and represents a potential future therapeutic avenue for the treatment of diabetic cardiomyopathy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

Adropin was synthesized by the University of Pittsburgh Peptide and Peptoid Synthesis Core. Due to space restrictions, we could not reference the important work of several groups in this field. We apologize to the authors of these works for any omissions.

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HIGHLIGHTS

- Long-term exposure to a high fat diet can promote metabolic dysfunction in the heart and reduce cardiac substrate flexibility.
- Adropin is a nutrient-responsive hormone shown to restore glucose oxidation in the skeletal muscle of diabetic mice.
- We show that acute treatment of high fat diet-induced obese mice with adropin restores cardiac glucose oxidation *in vivo*.
- Adropin improves pyruvate dehydrogenase activity in obese mice, which may be linked to reduced inhibitory lysine acetylation.

Figure 1- Regulation of cardiac fuel utilization in normal and obese mice by adropin.

(A) Schematic of adropin injection protocol. Mice received either vehicle (saline) or adropin (450 nmol/kg) by intraperitoneal injection over the course of three days. Following injection on the third day, mice were fasted for 6 hours and subjected to hyperinsulinemic-euglycemic clamps with U-¹³C glucose. (B-C) Body weights were significantly different in HF and HF +Adr animals relative to the chow group before and after catheter implantation. (D-E) Both HF and HF+Adr mouse groups showed significant hyperinsulinemia following a 6 hour fast relative to chow fed mice, but displayed no change in plasma glucose. (F-H) The glucose

infusion rate to maintain euglycemia was significantly reduced in both HF groups, and despite matched infusion levels during the clamp, HF and HF+Adr animals displayed modestly increased plasma insulin and glucose levels relative to chow mice, indicating hepatic insulin resistance in both HF groups. (I) The relative contribution of glucose oxidation to TCA cycle activity was significantly reduced in HF animals, and this was rescued by serial adropin injections. $N = 5-7$, $* = P < 0.05$ relative to chow. Data are mean $\pm$ SEM, with statistical testing by one-way ANOVA followed by Dunnett's multiple comparison post-hoc test. HF = High Fat, HF+Adr = High Fat plus Adropin.

Figure 2- Adropin treatment regulates the acetylation status and activity of Pyruvate Dehydrogenase.

(A) Treatment with adropin restored pyruvate dehydrogenase activity in the hearts of obese mice *in vitro*. (B-C) However, treatment with adropin did not significantly reduce *Pdk4* expression or pyruvate dehydrogenase (PDH) phosphorylation (S293) in mice *in vivo*. (D) Treatment with adropin led to a significant decrease in the cardiac abundance of the mitochondrial acetyltransferase GCN5L1 in HF animals. (E) A reduction in PDH acetylation, previously shown to inhibit enzymatic activity, was found after adropin treatment. A non-specific band of higher molecular weight is denoted by an asterisk, with the lower band at 43 kDa representing the PDH IP. N = 4-7, * = $P < 0.05$ relative to chow. (F) Acetylation of PDH is negatively correlated with pyruvate oxidation activity using linear regression analysis. N = 12. (G-I) Adropin treatment (1 $\mu\text{g/mL}$ for 24 hours) significantly stimulated PDH activity in rat H9c2 cells transfected with control empty vector. This increase was greatly attenuated in cells over-expressing GCN5L1. N = 4, * = $P < 0.05$

relative to vehicle-treated empty vector cells. Data are mean $\pm$ SEM, with statistical testing by one-way ANOVA followed by Dunnett's multiple comparison post-hoc test. HF = High Fat, HF+Adr = High Fat plus Adropin, Veh = Vehicle, Adr = Adropin, o/e = over-expressed GCN5L1 (~17 kDa), endo = endogenous GCN5L1 (~15 kDa).

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