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Translational

Adropin regulates cardiac energy metabolism and improves cardiac function and efficiency

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ABSTRACT

Background: Impaired cardiac insulin signalling and high cardiac fatty acid oxidation rates are characteristics of conditions of insulin resistance and diabetic cardiomyopathies. The potential role of liver-derived peptides such as adropin in mediating these changes in cardiac energy metabolism is unclear, despite the fact that in skeletal muscle adropin can preferentially promote glucose metabolism and improve insulin sensitivity.
Objectives: To determine the influence of adropin on cardiac energy metabolism, insulin signalling and cardiac efficiency.
Methods: C57Bl/6 mice were injected with either vehicle or a secretable form of adropin (450 nmol/kg, i.p.) three times over a 24-h period. The mice were fasted to accentuate the differences between animals in adropin plasma levels before their hearts were isolated and perfused using a working heart system. In addition, direct addition of adropin to the perfusate of *ex vivo* hearts isolated from non-fasting mice was utilized to investigate the acute effects of the peptide on heart metabolism and *ex vivo* function.
Results: In contrast to the observed fasting-induced predominance of fatty acid oxidation as a source of ATP production in control hearts, insulin inhibition of fatty acid oxidation was preserved by adropin treatment. Adropin-treated mouse hearts also showed a higher cardiac work, which was accompanied by improved cardiac efficiency and enhanced insulin signalling compared to control hearts. Interestingly, acute adropin administration to isolated working hearts also resulted in an inhibition of fatty acid oxidation, accompanied by a robust stimulation of glucose oxidation compared to vehicle-treated hearts. Adropin also increased activation of downstream cardiac insulin signalling. Moreover, both *in vivo* and *ex vivo* treatment protocols induced a reduction in the inhibitory phosphorylation of pyruvate dehydrogenase (PDH), the major enzyme of glucose oxidation, and the protein levels of the responsible kinase PDH kinase 4 and the insulin-signalling inhibitory phosphorylation of JNK (p-T^{183}/Y^{185}) and IRS-1 (p-S^{307}), suggesting acute receptor- and/or post-translational modification-mediated mechanisms.
Conclusions: These results demonstrate that adropin has important effects on energy metabolism in the heart and may be a putative candidate for the treatment of cardiac disease associated with impaired insulin sensitivity.

1. Introduction

Several secreted peptides have been shown to mediate whole body energy homeostasis, lipid metabolism and maintenance of insulin sensitivity [1–4]. For instance, adipokines, secreted by the adipose tissue, act as paracrine/endocrine hormones to mediate energy metabolism and have been extensively studied in the setting of obesity and diabetes [5]. However, less attention has been given to the potential metabolic actions of liver-secreted metabolic factors such as adropin [6]. Adropin is a secretable peptide initially identified in the liver and is encoded by the Energy Homeostasis Associated gene (Enho) [6]. Circulating adropin levels are closely related to nutritional and metabolic cues in the body.

Abbreviations: ACC, acetyl-CoA carboxylase; Akt, protein kinase B; AMPK, 5′ AMP-activated protein kinase; AS160, Akt substrate of 160 kDa; BSA, bovine serum albumin; CD36, cluster of differentiation 36; CoA, Coenzyme A; CPT, carnitine palmitoyltransferase; Enho, energy homeostasis associated gene; ERK1/2, extracellular signal-regulated kinases 1 & 2; FOXO1, forkhead box protein O1; GLUT, glucose transporter; GPR19, G-protein coupled receptor 19; GSK3β, glycogen synthase kinase 3 beta; IRS-1, insulin receptor substrate 1; JNK, c-Jun NH2-terminal kinase; LVDP, left ventricular developed pressure; PDH, pyruvate dehydrogenase; PDK4, pyruvate dehydrogenase kinase 4; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARα, peroxisome proliferator-activated receptor alpha; SIRT1, Sirtuin 1.

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For example, liver adropin expression decreases with diet- or genetically-induced obesity [6]. Additionally, the expression and plasma levels of adropin are increased by feeding and suppressed by fasting [6,7]. In skeletal muscles, adropin has been proposed to have a role in mediating energy metabolism through preferentially promoting glucose oxidation over fatty acid oxidation [8]. This is associated with improved insulin sensitivity in mice with diet-induced obesity [9]. This insulin sensitizing effect of adropin is thought to be mediated by an increase in glucose oxidation through activation of pyruvate dehydrogenase (PDH), by lowering the protein levels of it inhibitory kinase PDH kinase 4 (PDK4), while also inhibiting sarcolemmal and mitochondrial fatty acid uptake at a transcriptional level through decreasing the protein levels of the fatty acid transporter CD36, and carnitine palmitoyltransferase 1 (CPT I) [8,9].

Myocardial energy metabolism is profoundly altered in many heart pathologies including heart failure and diabetic cardiomyopathy [10]. In diabetes the heart is more reliant on fatty acid oxidation for energy production and displays impairment of glucose uptake and insulin signalling, whereas failing hearts show a general impairment of mitochondrial oxidative phosphorylation with an increased dependence on glycolysis [11]. These alterations in cardiac energy metabolism contribute to the severity of heart disease [11,12]. Clinically, reduced serum levels of adropin are associated with several cardiovascular conditions including endothelial dysfunction, heart failure, acute myocardial infarction, coronary atherosclerosis, and cardiac syndrome X, and represent an independent risk factor and predictor of most of these conditions [13]. Consequently, a relationship between adropin levels and cardiovascular energy metabolism and health is most likely to exist and warrants adequate investigation.

Whether adropin alters cardiac energy metabolism in the heart is unclear, as are the potential mechanisms by which it might mediate energy metabolism. Understanding the metabolic effects of adropin may not only provide diagnostic tools for early detection of cardiac pathologies but may also help devise novel therapies aiming at alleviating the metabolic disturbances contributing to the progression of heart disease. Therefore, the aim of this study was to investigate the effects of adropin administration on cardiac energy metabolism and function with the hypothesis that adropin causes a switch of myocardial energy metabolism towards glucose oxidation, while improving insulin sensitivity, thus improving the metabolic profile and cardiac efficiency.

2. Methods

2.1. Animals and materials

Male C57BL/6 mice (7–10 weeks of age) were obtained from Charles River laboratories (Wilmington, MA, USA) and used in the study. The animals were given free access to regular chow diet (Harlan Teklad, Madison, WI, USA) unless a fasting protocol was applied as explained below. All animals were randomized to one of the experimental groups explained below in study protocols. The injectable adropin peptide used in our study (adropin$^{34–76}$) is compatible with and native to the murine biology as the Enho mRNA sequence encodes a 76 aa open reading frame (ORF) that is identical between humans, rats, and mice, among other species [6]. Therefore, all animal studies were approved by the University of Alberta Health Sciences Animal Welfare Committee and conform to the guidelines of the Canadian Council of Animal Care, Alberta. A full list of materials is provided in the Supplementary information file.

2.2. Study protocol

The animals utilized in this study to evaluate cardiac functional and metabolic changes with adropin administration were subjected to one of the following two protocols.

2.2.1. In vivo adropin

Mice received three intraperitoneal (IP) injections of 450 nmol/kg adropin$^{34–76}$ dissolved in 0.1% BSA normal saline or vehicle, over a 20–24 h period. This adropin administration protocol was previously used to induce metabolic actions in whole-body and skeletal muscles in mice [8,9]. All mice were fasted 4 h after the first treatment injection just before their dark cycle, and for a total of 16–20 h (Fig. 1A). This fasting protocol was adopted to accentuate the differences in plasma levels of adropin between the animals of the two experimental groups and to investigate adropin's metabolic effects under conditions of decreased metabolic flexibility and increased reliance of the heart on fatty acid oxidation for energy production as what is seen in diabetes and obesity [8,9] that is similarly seen with fasting [11]. At the end of fasting period, the mice were euthanized using 12 mg sodium pentobarbital IP injections followed by isolating and perfusing the heart using a working heart preparation as described previously [14] [15,16] for 30 min without insulin, followed by 30 min with 100 μU/ml insulin. A schematic view of the *in vivo* protocol is shown in Fig. 1A.

2.2.2. Ex vivo adropin

To examine if adropin had acute metabolic/functional effects on the heart, we also acutely perfused hearts from normally fed animals. These animals did not receive treatments before euthanasia. Hearts were perfused using the *ex vivo* working heart system as described above, with the modification that during the whole perfusion protocol, 100 μU/ml insulin was present in the perfusate as well as either adropin$^{34–76}$ (2 nM, equivalent to 10 ng/ml), a slightly elevated yet physiologically relevant concentration of adropin, or vehicle. A schematic view of the *ex vivo* protocol is shown in Fig. 4A.

2.3. Heart perfusions

In addition to the specific treatment as explained above, the isolated working-heart perfusate consisted of a modified Krebs–Henseleit bicarbonate (KHB) solution containing in mM (118.5 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 25 $NaHCO_3$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 5 glucose, and 0.8 palmitate bound to 3% bovine serum albumin. Trace amounts of radiolabeled [5-^{3}H] glucose, [U-^{14}C] glucose, or [9, 10-^{3}H] palmitate were added to the perfusate to assess rates of glycolysis, glucose oxidation, and palmitate oxidation, respectively. The perfusate was continuously oxygenated with a gas mixture of 95% O_2 and 5% CO_2. Two metabolic rates were determined simultaneously by quantitative collection of $^{14}CO_2$ and ^3H_2O produced by the hearts from metabolizing the respective energy substrates. The dry/wet tissue ratio was determined and metabolic rates were represented as nmol per g dry weight per min (nmol·g dry wt^{-1}·min^{-1}) [15,16].

Cardiac function was assessed in isolated working hearts using a MP100 system from AcqKnowledge (BIOPAC Systems, Inc.). This included assessing heart rate, peak systolic pressure, left ventricular developed pressure, cardiac output, and aortic output [17]. Cardiac work was determined as a function of cardiac output and peak systolic pressure. Cardiac efficiency was calculated as the ratio of cardiac work to total ATP production rates, which in turn was obtained from energy substrate metabolic rates. At the end of each perfusion, the heart ventricles were clamp-frozen in liquid nitrogen then stored at −80 °C for subsequent biochemical examination.

2.4. Western blotting

Protein levels and phosphorylation were assessed in heart tissue using immunoblotting, as described previously [18]. For details see the Supplementary information file.

2.5. Protein acetylation assay

Specific protein acetylation was assessed using an immunoprecipitation (IP) method as previously described [19] with minor modification. For details see the Supplementary information file.

2.6. Glucose tolerance test

An intraperitoneal glucose tolerance test was conducted on fasted mice administered adropin or vehicle using a dose of 2 g glucose/kg as previously described [20]. For details see the Supplementary information file.

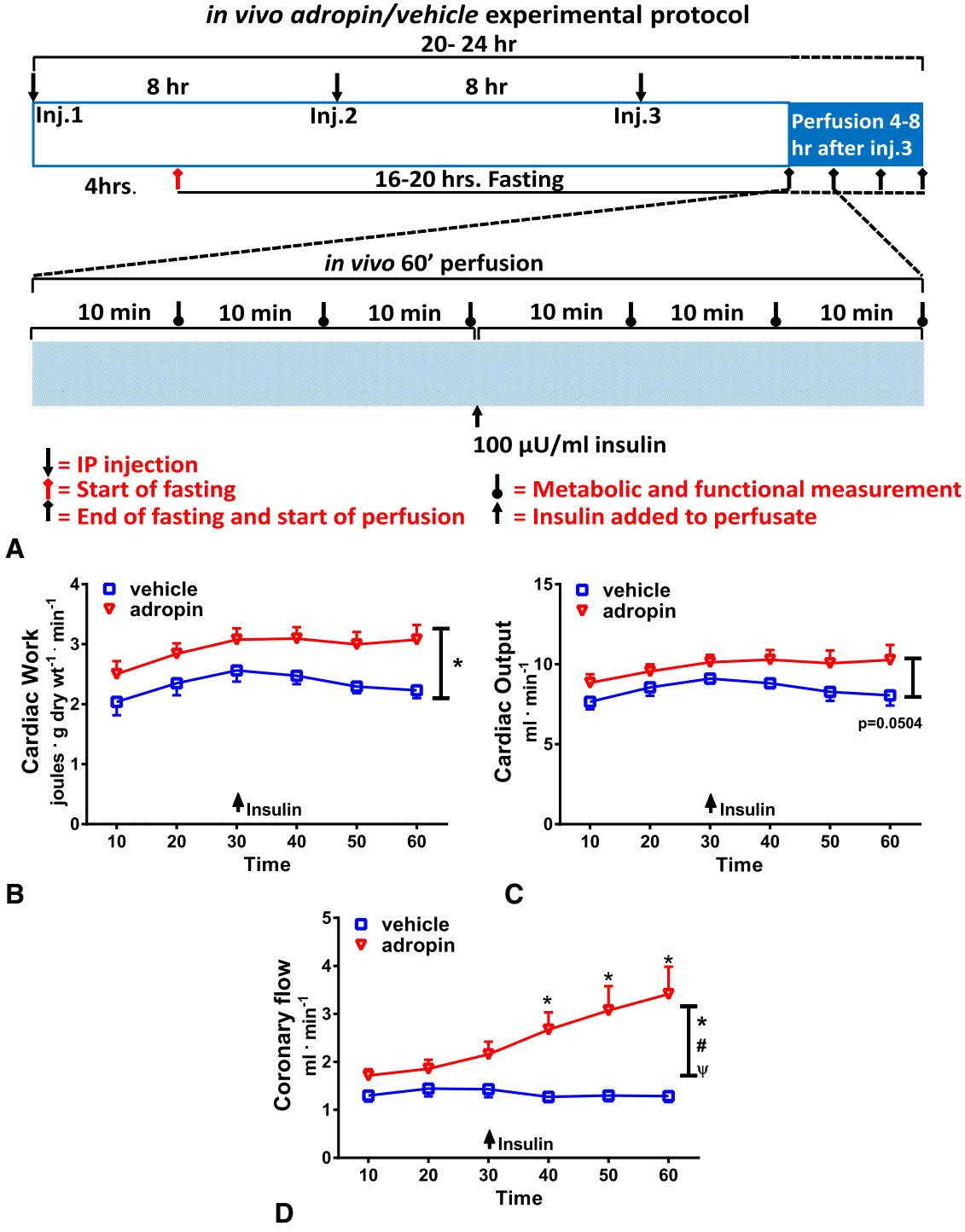

Fig. 1. *In vivo* adropin administration improves cardiac function in fasted mice. (A) A diagram showing the experimental protocol of *in vivo* adropin/vehicle injections followed by isolated working hearts perfusions. Functional and metabolic assessment of working heart perfusions (60 min) of hearts isolated from fasting C57BL/6 mice that were injected three times with intraperitoneal 450 nmol/kg adropin[34–76] or vehicle over a 20–24 h period: (B) cardiac work, (C) cardiac output, and (D) coronary flow, Cardiac functional parameters data were recorded using an MP100 system from AcqKnowledge (BIOPAC Systems, Inc.). Data provided were used to calculate cardiac work and coronary flow as explained in experimental procedures. $^{*}p \ll 0.05$ (treatment), $^{\#}p \ll 0.05$ (time), $^{\psi}p \ll 0.05$ (interaction), 2-way ANOVA with repeated measures. * on individual points show significance at the specific time point with Bonferroni's test. All values represented as mean ± SEM, n = 7. IP, intraperitoneal.

2.7. Statistical analysis

Data are expressed as mean ± SEM. Statistical significance was determined using paired, unpaired *t*-test, 2-ANOVA, or 2-ANOVA with repeated measures, followed by Bonferroni post-hoc test whenever appropriate. Differences were deemed significant if $p \ll 0.05$.

3. Results

3.1. Adropin administration to fasting mice improves cardiac function and efficiency while increasing insulin's inhibitory effect on cardiac fatty acid oxidation

As expected in these lean mice, not displaying insulin resistance or impaired glucose homeostasis, whole body glucose tolerance was not different between the two groups (Supplementary Fig. 1). Interestingly, treatment with adropin improved *ex vivo* cardiac function, as evidenced by enhanced cardiac work especially upon the introduction of insulin to the perfusate (Fig. 1B). This was partially a result of increased cardiac output which was higher in adropin's group, although not reaching statistical significance (Fig. 1C). Additionally, there was a significant increase in coronary flow (Fig. 1D), which may have contributed to the enhanced cardiac work.

Despite fasting, the effect of insulin on stimulating cardiac glucose oxidation rates was seen in both vehicle and adropin-treated mice (Fig. 2A). In contrast, hearts from vehicle-treated fasted mice, lacked a significant inhibitory effect of insulin on palmitate oxidation (Fig. 2B), that normally exist in fed mice. However, the inhibitory effect of insulin on fatty acid oxidation was preserved in hearts from adropin-treated mice (Fig. 2B). This suggests enhanced myocardial insulin sensitivity with adropin despite the partial masking effect of fasting that largely suppressed glucose oxidation in all hearts. Glycolysis rates were also not different between hearts from adropin-treated mice and vehicle-treated mice (Fig. 2C), suggesting that the insulin sensitizing effects of adropin on glucose metabolism are independent of glycolysis.

The relative contribution of fatty acid oxidation to ATP production was decreased by insulin in hearts from adropin-treated mice (Fig. 2D) which resulted in a moderate decrease in total ATP produced (not shown) to provide a higher cardiac work as shown in Fig. 1B above, thus leading to a rise in cardiac efficiency in the hearts from adropin-treated mice (Fig. 2E) which in turn is defined as the amount of cardiac work per ATP produced. Together, these results suggest that three injections of adropin over 24 h were enough to promote *ex vivo* cardiac function with an enhancement of insulin's inhibitory effect on fatty acid oxidation, ultimately resulting in a higher cardiac efficiency.

3.2. In vivo 24 h treatment with adropin decreases the phosphorylation of pyruvate dehydrogenase and protein levels of pyruvate dehydrogenase kinase 4

Based on our functional and metabolic data, we investigated possible variations in levels and phosphorylation status of proteins known to

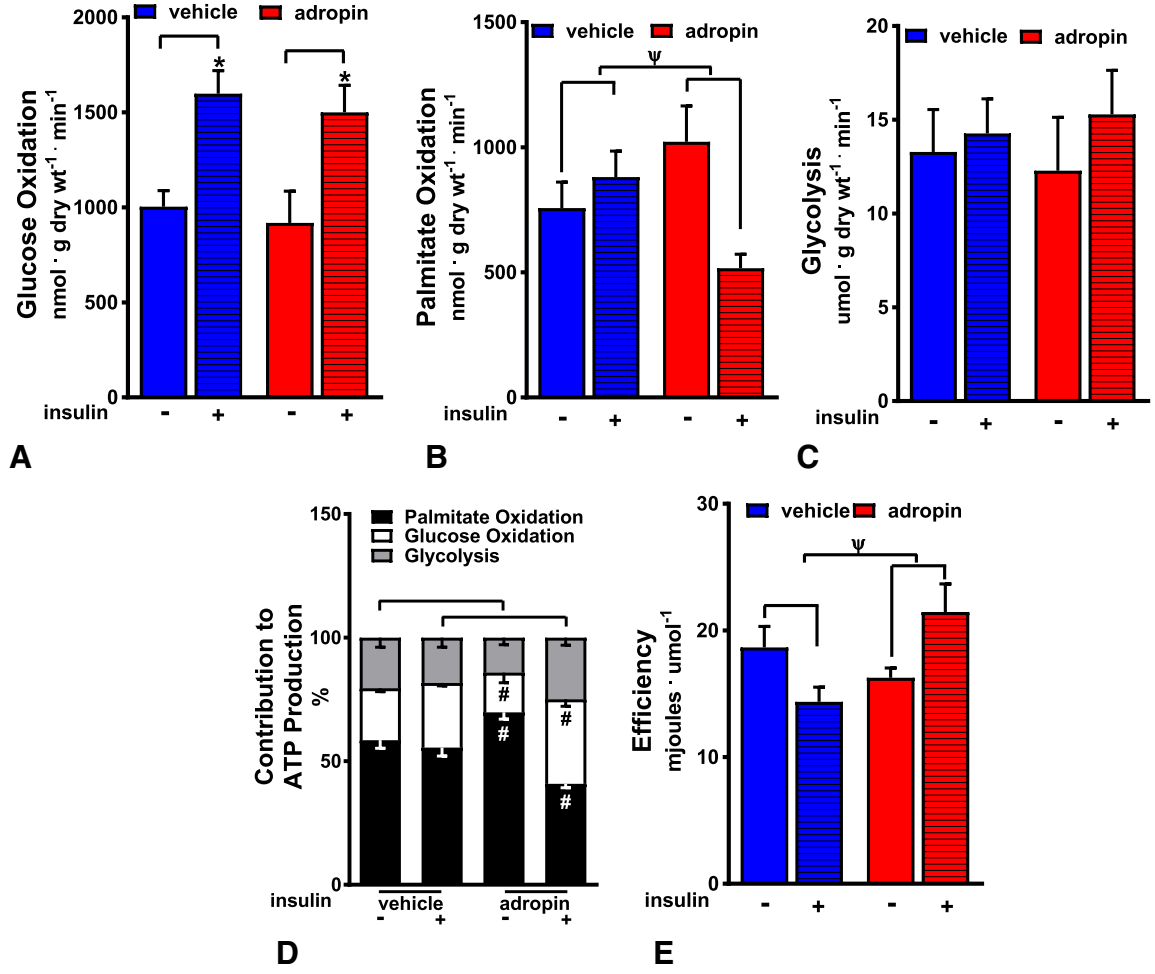

Fig. 2. *In vivo* adropin enhances insulin-induced inhibition of cardiac fatty acid oxidation and increases efficiency. (A) Glucose oxidation; (B) palmitate oxidation; (C) glycolysis; (D) percent contribution of catabolic pathway to ATP production and (E) cardiac efficiency. Insulin (100 μU/ml) was added at 30 min during heart perfusions as described in methods. $^{*}p < 0.05$, paired *t*-test. $^{\psi}p \ll 0.05$ (interaction), 2-way ANOVA, $^{\#}p \ll 0.05$ *vs.* vehicle of same insulin treatment, 2-way ANOVA followed by Bonferroni's test. All values represented as mean ± SEM, n = 5–8.

regulate energy metabolic pathways. Accordingly, we explored some mechanisms previously suggested to mediate adropin effects in skeletal muscles at a translational level [8,9]. Contrary to the proposed paradigm in skeletal muscles, we did not see changes in protein levels of CD36, PPARα, PGC-α, or CPT Ib in the heart (Fig. 3A and B). However, consistent with findings in skeletal muscles [9] and cultured H9c2 cardiomyocytes [21], PDK4 protein levels were significantly reduced in the hearts of adropin-treated mice (Fig. 3A and B). It was suggested adropin's metabolic effects are mediated in skeletal muscles through the inhibitory acetylation control of the transcriptional coactivator PGC-1α with subsequent effects on PDK4 and CPT levels [8,9]. However, we could not reproduce such acetylation changes (Supplementary Fig. 2) with adropin in the heart.

As AMPK and ACC2 play a major role in the regulation of mitochondrial uptake of long-chain fatty acyl groups, and hence fatty acid oxidation, through the malonyl-CoA axis, we investigated the phosphorylation status of these enzymes. The well-established signalling pathway involves a stimulatory phosphorylation of AMPK which, in turn, phosphorylates and inhibits ACC2, the enzyme responsible for the production of the endogenous fatty acid oxidation inhibitor malonyl-CoA. However, neither of these two enzymes were differently phosphorylated (Fig. 3C and D), suggesting that other mechanisms may account for adropin's metabolic effects. In contrast, the inhibitory phosphorylation of PDH E1-alpha subunit at S^{293}, the rate limiting and major enzyme in the glucose oxidation pathway, appears to be reduced by adropin injections (Fig. 3C and D), which is consistent with the drop in PDK4 protein levels. These results suggest that in the heart the metabolic effects associated with adropin administration over a 24-h period are at least partially explained by variable protein expression of PDK4 and its subsequent phosphorylation of PDH.

3.3. Acute administration of adropin stimulates glucose oxidation with a corresponding inhibition of palmitate oxidation

Since we did not see effects of adropin injections on protein levels of several metabolic regulatory components that were previously suggested to mediate adropin's effects on skeletal muscle energy metabolism [8,9], we went on to examine whether adropin has any acute metabolic and/or functional effects on the heart that are not dependent on protein expression. This was

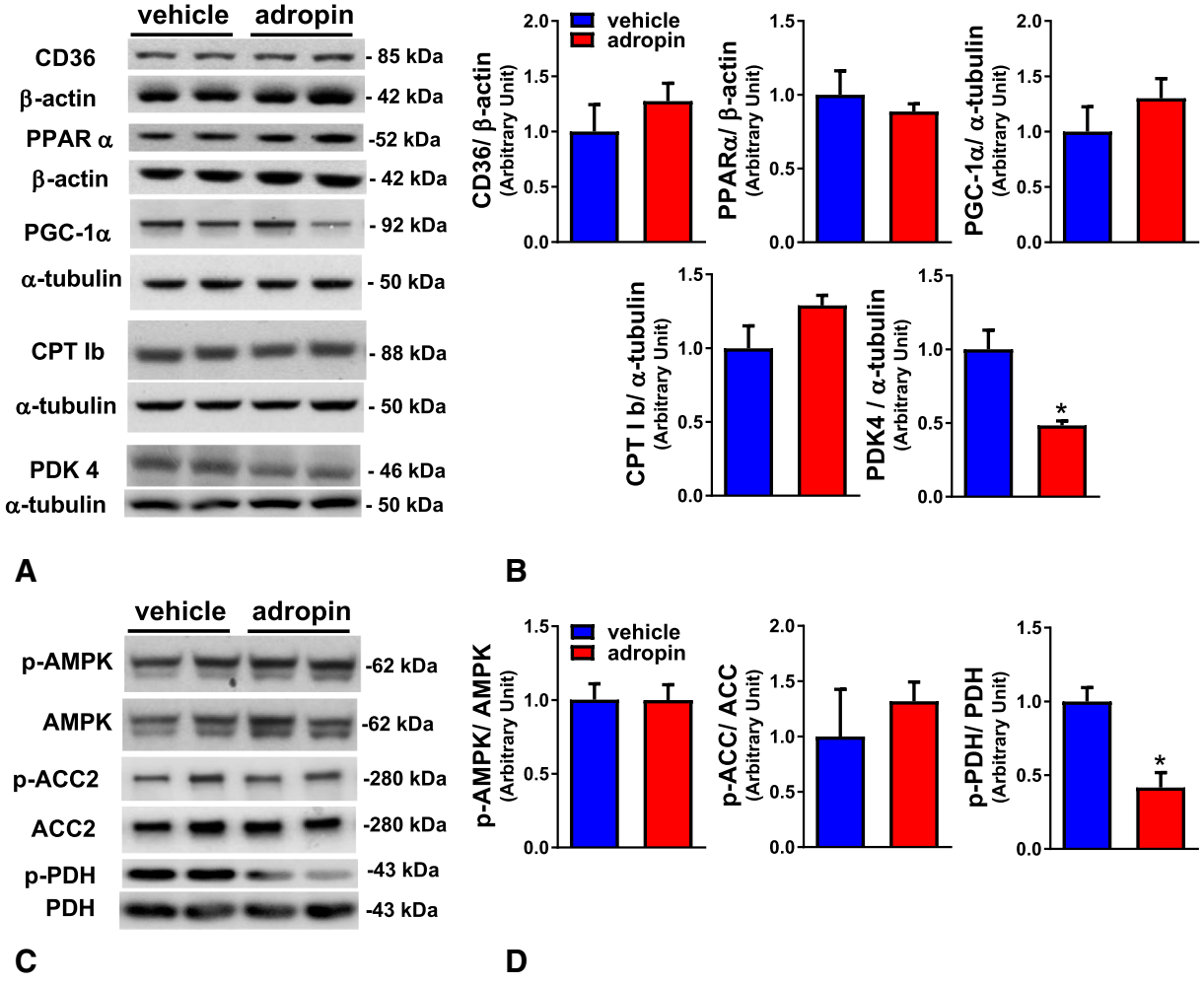

Fig. 3. *In vivo* adropin decreases cardiac PDK4 protein expression. (A) Western blots of key enzymes involved glucose and fatty acid oxidation, CD36, PPARα, PGC-1α, CPT I B, and PDK4, indicating protein levels. α-Tubulin or β-actin was used as protein loading controls of the respective protein. (B) Quantification of data presented in (A). (C) Western blots showing the phosphorylation of the key enzymes involved glucose and fatty acid oxidation p-PDH S^{293}, p-AMPK T^{172}, and p-ACC2 S^{1200}, correlating to their activity. The phosphorylated forms of PDH, AMPK, and ACC2 are normalized for the total protein level of the respective enzyme. (D) Quantification of data presented in (C). Western blot samples were prepared from heart ventricles clamp-frozen at the end of the 60 min isolated heart perfusion of hearts isolated from fasting C57BL/6 mice that were injected three times with intraperitoneal 450 nmol/kg adropin$^{34-76}$ or vehicle over a 20–24 h period. *$p<0.05$. All values represented as mean ± SEM, $n = 5–6$. ACC, acetyl-CoA carboxylase; AMPK, 5′ AMP-activated protein kinase; CD36, cluster of differentiation 36/fatty acid translocase; CPT, carnitine palmitoyltransferase; PDH, pyruvate dehydrogenase; PDK4, pyruvate dehydrogenase kinase 4; PPARα, peroxisome proliferator-activated receptor alpha; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha.

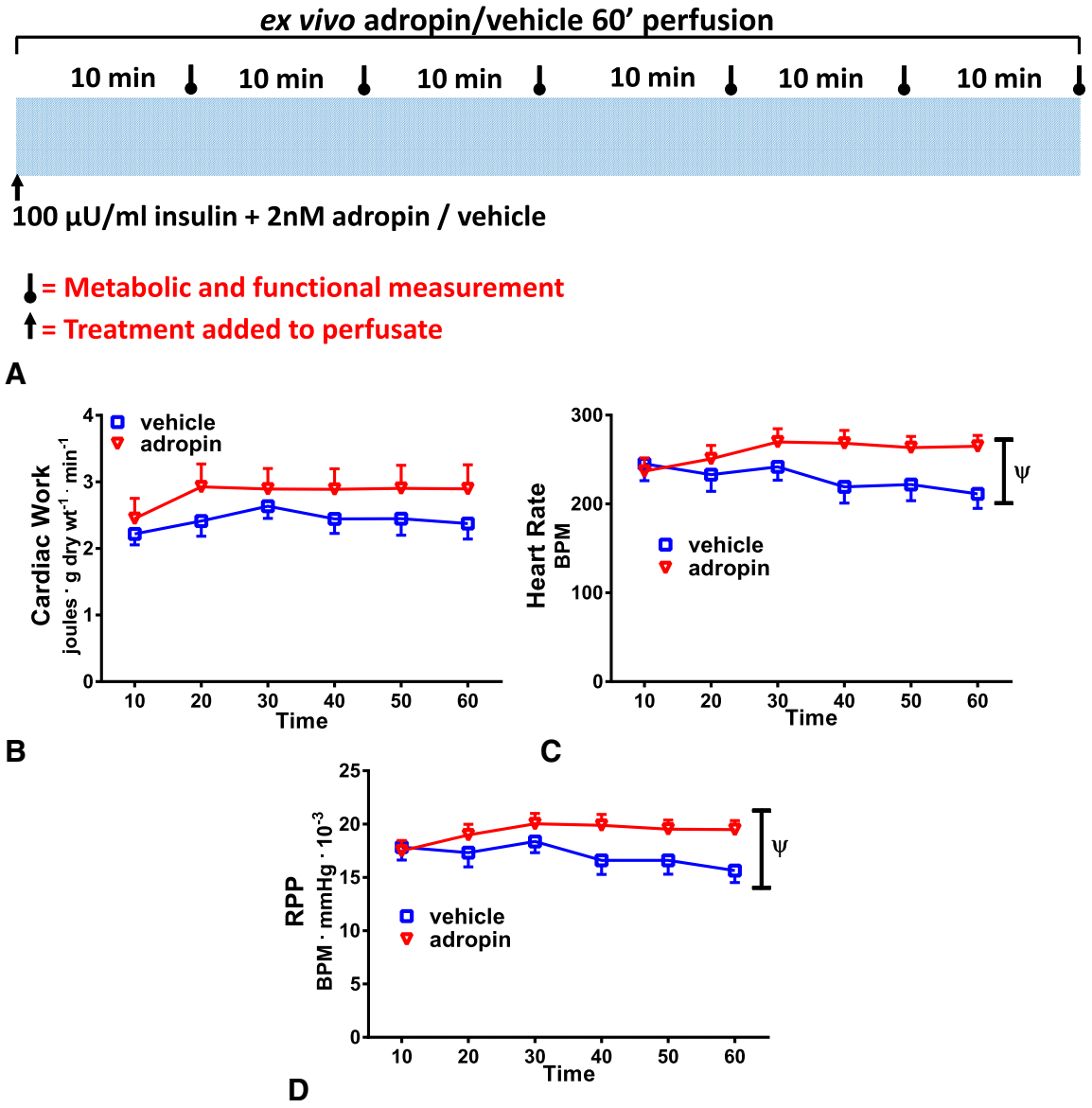

Fig. 4. *Ex vivo* adropin does not alter cardiac function. (A) A diagram showing the experimental protocol of *ex vivo* adropin/vehicle working hearts perfusions as explained in methods. Functional and metabolic assessment of working heart perfusions (60 min) of hearts isolated from non-fasting C57BL/6 mice and then perfused with 2 nM adropin$^{34-76}$ or vehicle: (B) Cardiac work; (C) heart rate, and (D) rate pressure product (RPP). Cardiac functional parameters data were recorded using an MP100 system from AcqKnowledge (BIOPAC Systems, Inc.). Data provided were used to calculate RPP and cardiac work as explained in experimental procedures. $^{\psi}p \ll 0.05$ (interaction), 2-way ANOVA with repeated measures. All values represented as mean ± SEM, n = 14.

achieved through directly perfusing isolated working mouse hearts with adropin or vehicle (protocol is shown in Fig. 4A). Acute adropin-treatment did not have major effects on cardiac work (Fig. 4B), although there was a significant preservation of heart rate and rate-pressure product (RPP) over the duration of perfusion (Fig. 4C and D, respectively). Despite these minor effects on cardiac function, hearts perfused with adropin showed a significant enhancement of glucose oxidation accompanied by a corresponding decrease in palmitate oxidation (Fig. 5A and B, respectively) compared to control. Here again, there was no change in glycolysis rates (Fig. 5C), in support of the idea that adropin's effects are independent of glycolysis. The changes in glucose/fatty acid oxidation rates resulted in significant increases in the relative contribution of glucose oxidation to the production of ATP (Fig. 5D). Since these non-stressed hearts produced comparable total amount of ATP and magnitude of cardiac work, their cardiac efficiency did not differ from controls (Fig. 5E).

3.4. Insulin signalling is enhanced in the hearts of adropin-injected mice and in isolated hearts perfused with adropin

The acute and profound effect of adropin administration on stimulating cardiac glucose oxidation while inhibiting fatty acid oxidation, along with the limited changes in the expression of several proteins involved in energy metabolism following *in vivo* adropin administration, led us to the hypothesis that a post-translational mechanism(s) may actually be responsible for the effects of adropin on cardiac energy metabolism in the heart. We therefore measured several components of the insulin signalling pathway following both *in vivo* and *ex vivo* adropin treatments. As shown in Fig. 6, tissue lysates of heart ventricles from mice treated with *in vivo* adropin showed an enhanced stimulatory phosphorylation of Akt (p-Akt S^{473}), AS160 (p-AS160 T^{642}), and insulin receptor substrate-1 (p- IRS-1 Y^{628}), along with an increased inhibitory phosphorylation of GSK3β (p-GSK3β S^{9}) compared to vehicle controls ($p \ll 0.05$) (Fig. 6A and B), all indicating enhancement of insulin

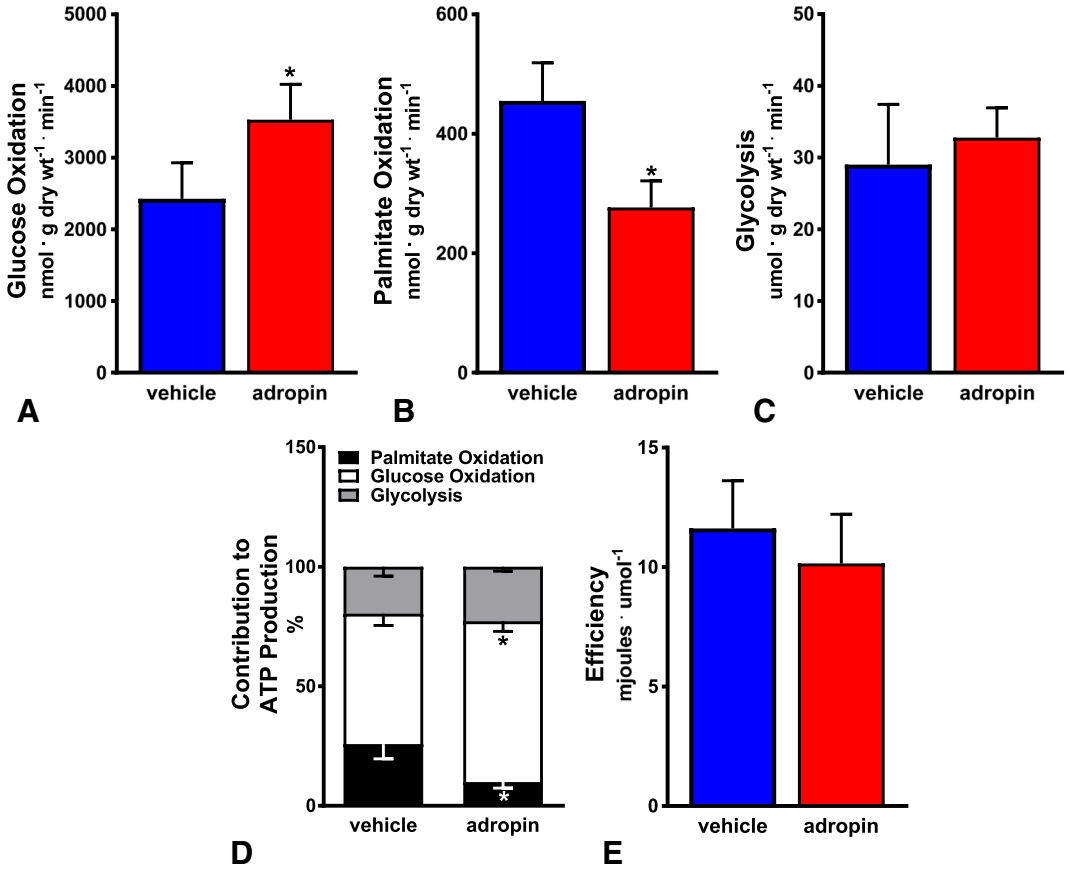

Fig. 5. *Ex vivo* adropin enhances cardiac glucose oxidation and inhibits fatty acid oxidation. (A) Glucose oxidation; (B) palmitate oxidation; (C) glycolysis; (D) percent contribution of the respective catabolic pathway to ATP production and; (E) cardiac efficiency. Insulin (100 μU/ml) was present from the beginning of heart perfusions as described in methods. *$p \ll 0.05$ compared to vehicle controls, unpaired *t*-test. All values represented as mean ± SEM, n = 6–14.

signalling. Similarly, *ex vivo* adropin also increased insulin signalling compared to vehicle controls, as evidenced by increased stimulatory phosphorylation of IRS-1 (p- IRS-1 Y^{628}) and Akt (p-Akt S^{473}), as well as by downstream inhibitory phosphorylation of GSK3β (p-GSK3β S^{9}) in *ex vivo* adropin hearts ($p \ll 0.05$), although not significantly changing AS160 phosphorylation (p-AS160 T^{642}) status (Fig. 7A and B). Of note, *ex vivo* adropin also showed reduced phosphorylation of PDH (Fig. 7A and B) as was seen with the *in vivo* protocol above. Of interest, a one-hour exposure of the mouse hearts to adropin in the perfusate resulted in reduction of PDK4 levels (Fig. 7C and D), which correlated well with the reduced phosphorylation of PDH. Taken together, adropin administration to mice or directly to the mouse heart stimulates insulin signalling and sensitivity in cardiac ventricular tissue.

3.5. Adropin reduces PDK4 protein levels and enhances insulin-IRS-1 pathway through mechanisms involving MAPKs and FOXO1 signalling

A negative regulator of IRS-1 is its phosphorylation at S^{307} residues in mouse tissue (S^{312} in the human) which is located at a domain involved in the binding of IRS-1 to the insulin receptor (IR), and when phosphorylated hinders this binding and subsequent insulin signalling [22]. We observed a decline in IRS-1 S^{307} phosphorylation with both *in vivo* and *ex vivo* adropin administration (Figs. 6A & B and 7A & B). This is also consistent with stimulation of insulin signalling and improved cardiac insulin sensitivity as evident in the above molecular and metabolic effects of adropin. One upstream kinase responsible for IRS-1 S^{307} phosphorylation is c-Jun NH_2-terminal kinase (JNK) mitogen-activated protein kinase [23], which displayed decreased phosphorylation (p-JNK T^{183}/Y^{185}) with both *in vivo* and *ex vivo* adropin administration protocols. This decreased phosphorylation was associated with inhibited activity of this kinase and consequently in line with decreased IRS-1 S^{307} phosphorylation (Figs. 6 and 7). FOXO1 and another mitogen-activated protein kinase, ERK 1/2, are both responsive to insulin stimulation and are known to regulate PDK4 expression [24,25]. FOXO1 is phosphorylated and inhibited by Akt [26]. We detected an increased phosphorylation of FOXO1 and ERK1/2 by both treatment protocols of adropin (Figs. 6C and D and 7C and D), which may account at least in part for the decline in PDK4 with adropin treatments. To summarize, *in vivo* adropin administration as well as acute *ex vivo* adropin in isolated hearts was associated with stimulation of insulin signalling pathways that was accompanied by inhibitory effects on FOXO1-PDK4 and JNK/IRS-1 S^{307} phosphorylation axis as well as activation of ERK1/2 pathway.

4. Discussion

Several peptides secreted by non-muscular tissues are known to affect energy homeostasis and insulin sensitivity in heart and skeletal muscle [1,27–29]. Dysregulated plasma levels and functions of certain adipokines including adiponectin, leptin as well as pro-inflammatory cytokines and resistin are associated with insulin resistance seen in obesity [28,29]. Here we show that a secreted form of adropin (*i.e.* adropin$^{34–76}$), which is naturally synthesized and secreted by the liver, can also induce significant modification of cardiac energy metabolism by increasing glucose oxidation and inhibiting fatty acid oxidation.

Decreased adropin plasma levels are associated with markers of insulin resistance [30], while heart failure is associated with elevated levels of plasma adropin [31] perhaps as an adaptive mechanism. Adropin has been proposed to have an important role in regulating skeletal muscle energy metabolism particularly in post-prandial conditions

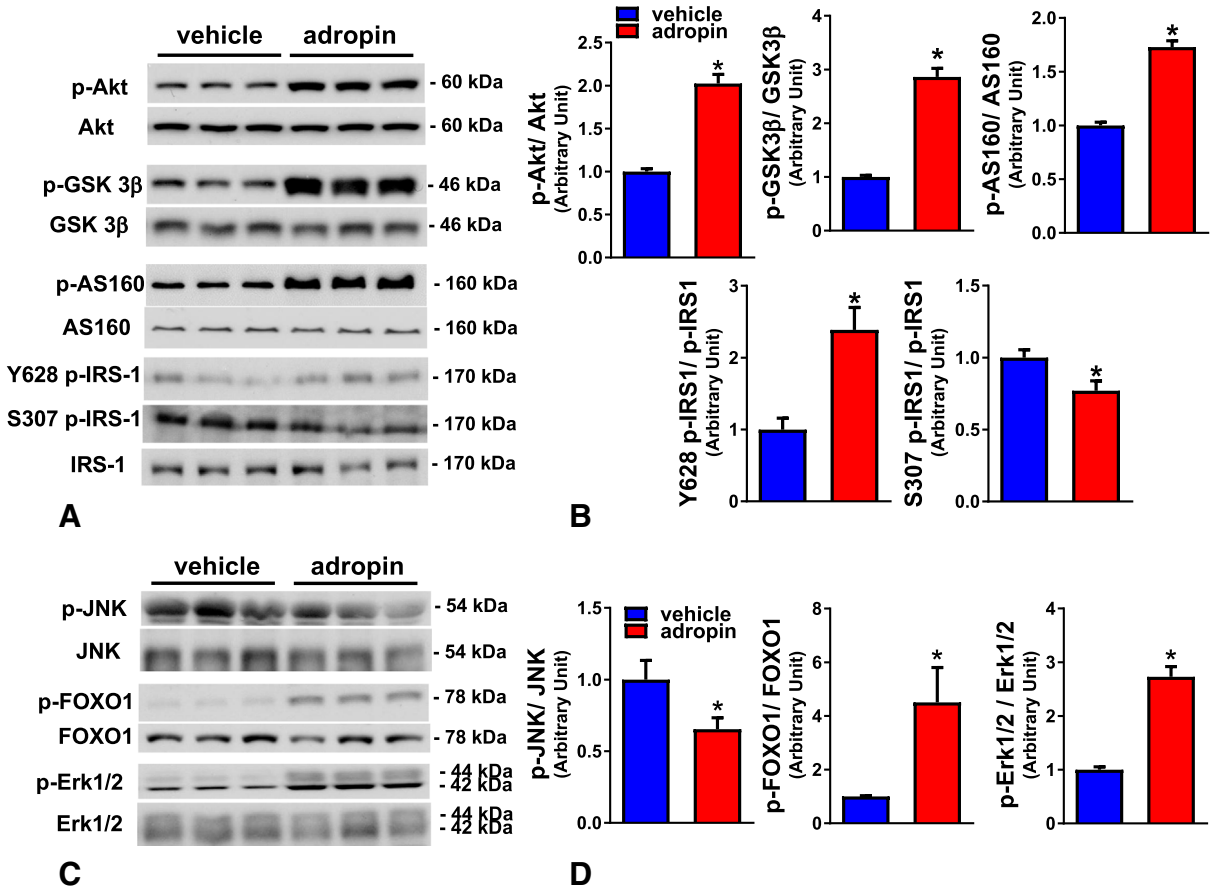

Fig. 6. *In vivo* adropin increases cardiac insulin sensitivity. (A) Western blots showing phosphorylation of key components of the insulin signalling pathway: p-Akt^{S473}, p-GSK3β^{S9}, p-AS160^{T642}, p-IRS-1^{Y628}, and p-IRS-1^{S307}, as surrogate markers of their activity. (B) Quantification of data presented in (A). (C) Western blots of proteins involved in regulation of PDK4 expression and insulin signalling, namely p-JNK$^{T183/Y185}$, p-FOXO1^{S256}, and p-ERK1/2$^{T202/T204}$. (D) Quantification of data presented in (C). The phosphorylated form of each protein is normalized for the total level of the same protein. Western blot samples prepared from heart ventricles clamp-frozen at the end of the 60 min isolated heart perfusion of hearts isolated from fasting C57BL/6 mice that were injected three times with intraperitoneal 450 nmol/kg adropin$^{34-76}$ or vehicle over a 20–24 h period. *p ≪ 0.05 *versus* vehicle control, unpaired t-test. All values represented as mean ± SEM, n = 5–6. Akt, protein kinase B; AS160, Akt substrate of 160 kDa; ERK1/2, extracellular signal-regulated kinases 1 & 2; FOXO1, forkhead box protein O1; GSK3β, glycogen synthase kinase 3 beta; IRS-1, insulin receptor substrate 1; JNK, c-Jun NH2-terminal kinase.

[6,8] and in obesity [9]. In these previous studies, either transgenic overexpression or exogenous administration of adropin improves the overall metabolic profile in animals with diet-induced obesity through better glucose tolerance and oxidation and insulin sensitivity [6,9]. The expression and plasma levels of adropin are increased during feeding and suppressed by fasting [6,7]. This may contribute to the flexibility that the myocardium displays in switching between energy substrates, from the fatty-acid reliant fasting state to the more metabolically flexible fed state, where glucose is more abundant in the circulation [11]. In general, and due to multiple reasons, fatty acids are considered less efficient as a source of energy to the myocardium [11,32,33]. Accordingly, current metabolic modulators are almost invariably aimed at enhancing glucose oxidation in relation to fatty acid metabolism [34,35].

Direct effects of adropin on cardiac energy substrate metabolism have not been clearly demonstrated. To examine the effects of adropin on cardiac energy metabolism we utilized a fasting protocol applied to mice that were injected with adropin or vehicle. Injected adropin has been shown to enhance whole body glucose clearance in diet-induced obesity whereas adropin overexpressing transgenic mice show improved glucose tolerance even on regular chow diet [6]. The discrepancy between those lean transgenic mice and our wild type lean mice is mostly due to the difference in the length of exposure of insulin-sensitive tissues to a constitutive overexpression and oversecretion of adropin as opposed to a transient increase in its levels over 24 h. Although we did not use in this preliminary study an experimental model of diabetes, which is a limitation in our study, our protocol allowed us to simulate the metabolic status of diabetic hearts by creating a fasting-induced elevation of fatty acid oxidation and metabolic inflexibility accompanied by inhibited rates of glucose oxidation. This proved useful in evaluating adropin for possible effects counteracting the fasting-associated energy metabolism profile akin to the insulin-resistant heart.

Our demonstration of direct beneficial effects of adropin on cardiac function may suggest useful implications and benefit in conditions of cardiac disease associated with impaired insulin sensitivity. Indeed, previous studies have demonstrated an association between cardiac insulin resistance and the severity of heart failure [11,36–40]. As a result, an improved cardiac function in adropin treated mice may be occurring secondary to improved cardiac insulin sensitivity. However, this study represents a preliminary evaluation of actions of adropin on cardiac function and future investigations on diseased hearts are warranted. The exact mechanism of improved cardiac function, especially with insulin stimulation, is not clear, although this may be related to an increased cardiac efficiency with *in vivo* adropin (Fig. 2F), a finding consistent with increased cardiac insulin sensitivity, increased glucose oxidation, and inhibition of fatty acid oxidation (which is a less efficient energy substrate compared to glucose) [11,17,33,41].

The mechanism by which adropin has previously been proposed to stimulate glucose oxidation in skeletal muscles or stimulate mitochondrial respiration in H9c2 cells involved stimulating PDH activity by down-regulating the protein expression of its inhibitory kinase PDK4 [8,9]. Our results are in agreement with these previous studies and go

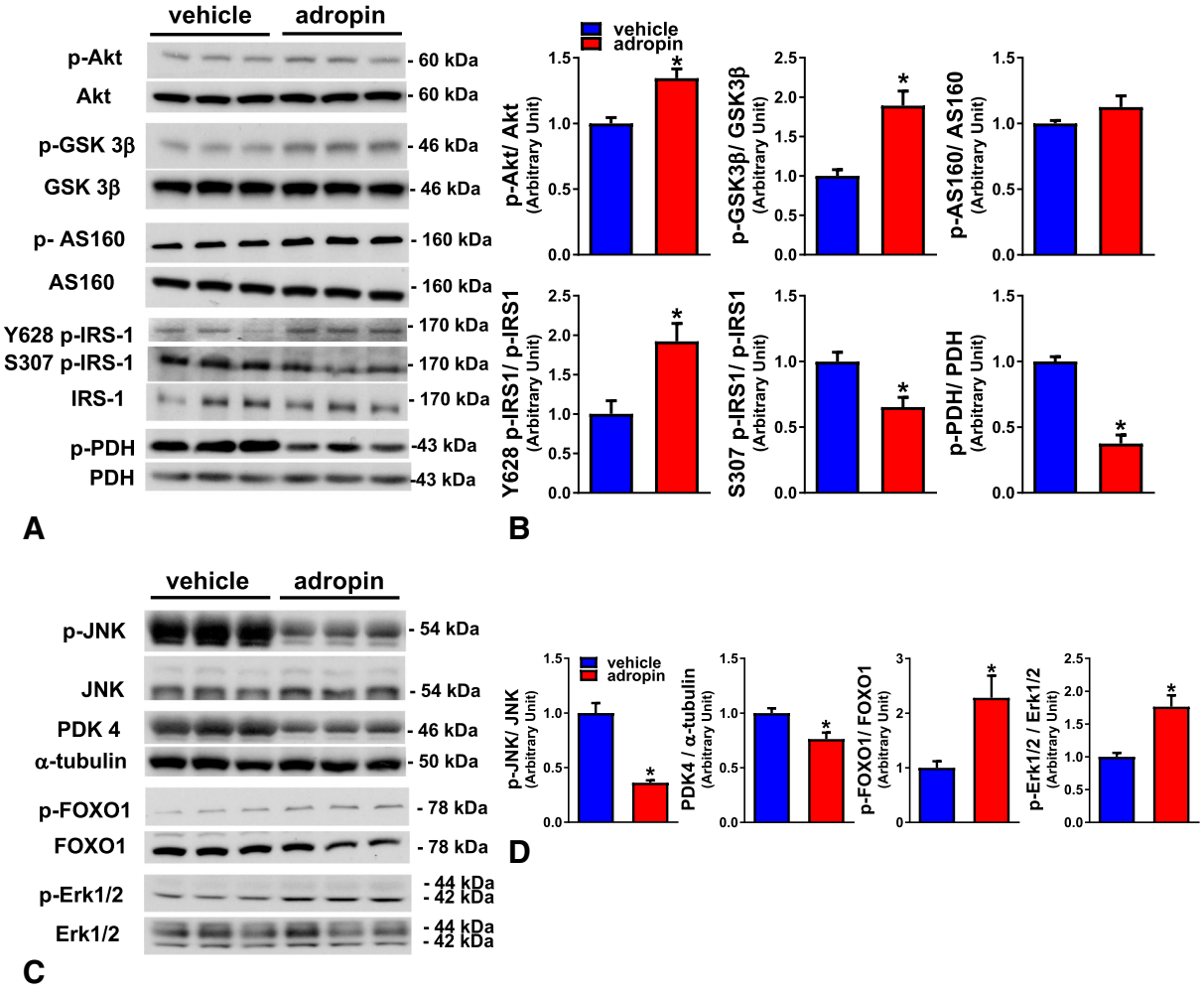

Fig. 7. *Ex vivo* adropin increases cardiac insulin sensitivity. (A) Western blots showing phosphorylation of key proteins in the insulin signalling pathway and glucose metabolism: p-Akt^{S473}, p-GSK3β^{S9}, p-AS160^{T642}, p-IRS-1^{Y628}, p-IRS-1^{S307}, and p-PDH S^{293} as surrogate markers of their activity. (B) Quantification of data presented in (A). (C) Western blots of PDK4 and proteins involved in the regulation of its expression as well as in regulation of insulin signalling, namely p-JNK$^{T183/Y185}$, p-FOXO1^{S256}, and p-ERK1/2$^{T202/T204}$. (D) Quantification of data presented in (C). The phosphorylated form of each protein is normalized for the total level of the same protein. WB samples were made from heart ventricles clamp-frozen at the end of the 60 min isolated heart perfusion of hearts isolated from non-fasting C57BL/6 mice and then perfused with 2 nM adropin$^{34–76}$ or vehicle. *p ≪ 0.05 *versus* vehicle control, unpaired t-test. All values represented as mean ± SEM, n = 5–8. Akt, protein kinase B; AS160, Akt substrate of 160 kDa; ERK1/2, extracellular signal-regulated kinases 1 & 2; FOXO1, forkhead box protein O1; GSK3β, glycogen synthase kinase 3 beta; IRS-1, insulin receptor substrate 1; JNK, c-Jun NH2-terminal kinase; PDH, pyruvate dehydrogenase; PDK4, pyruvate dehydrogenase kinase 4.

further to show a similar effect with acute adropin exposure on decreasing PDK4 expression. PDK4 levels are not only dependent on its rate of synthesis, but also its degradation by the mitochondrial protease Lon, and possible other proteases and ubiquitination pathways which contribute to its short life (t½ ~ 1 h) [42]. Consistently, a decline in PDK4 levels was achieved by the first hour of exposure of H9c2 cells to adropin [21]. On the other hand, inhibition of fatty acid oxidation was proposed to occur through decreasing the protein expression of the sarcolemmal fatty acid translocase, CD36, and of CPT I, the enzyme responsible for mitochondrial uptake and hence oxidation of activated long fatty acids [8,9]. In addition, it was suggested that these effects are mediated through the inhibitory acetylation control of the transcriptional coactivator PGC-1α with subsequent effects on CD36 and CPT levels [8,9]. However, we could not reproduce such protein level changes of CD36, CPT1b, and PGC-1α, which agrees with the lack of effect on these proteins in cultured cardiomyocytes incubated with adropin for 24 h. Acetylation changes with adropin were not reproduced either in our study (Supplementary Fig. 2). This suggests that the cardiac response to adropin may involve different mechanisms depending on the tissue affected and/or duration of treatment. Nonetheless, the phosphorylation of PDH was reduced by *in vivo* adropin injections as well as after acute *ex vivo* administration of adropin to the heart. The discrepancy of this latter finding compared to the lack of p-PDH changes by 4 h of adropin exposure in H9c2 cells [21] is unclear, but may stem from the fact that H9c2 cells are considered a more glycolytic type of cells that are not beating or subject to actual workloads and thus require minimal energy production and are less reliable on glucose oxidation and PDH activity.

Several components of cardiac insulin signalling were stimulated by adropin in our study, associated with increased glucose oxidation and/or reduced fatty acid oxidation (Fig. 6). These are consistent with the aforementioned studies on normal and insulin-resistant mouse skeletal muscles [8,9]. Noteworthy, in our *in vivo* treatment model, glucose oxidation was not increased with adropin in parallel with the reduced inhibitory phosphorylation of PDH. The exact reason behind this observation is unclear but could be due to the complexity of mechanisms regulating energy metabolism in general under fasting conditions which, *per se*, suppresses glucose oxidation. However, the observed reduction of fatty acid oxidation with adropin injections is important since conditions such as obesity and diabetes are associated with cardiac

insulin resistance and increased reliance on fatty acids for energy production. On the other hand, cardiac glycolysis rates were not changed by adropin which is consistent with previous studies using models of insulin resistance including heart failure and high fat diet, that showed reduction in glucose oxidation without changes in glycolysis [43,44]. Effects of adropin on gluconeogenesis and glycogenolysis were not investigated in our study as we focused on heart glucose and fatty acid oxidation, being the major energy sources for the heart, and due to the fact that the heart does not possess significant gluconeogenic capacity, secondary to the limited phosphoenolpyruvate carboxykinase (PEPCK) protein expression [45]. However, a recent study showed that adropin can reduce hepatic glucose production and improve insulin sensitivity in the liver [46].

The impressive acute adropin metabolic response in the absence of alterations in the expression of key metabolic proteins suggest a mechanism of action involving a possible ligand (adropin)-receptor interaction, resulting in the post-translational modifications of components of the insulin signalling pathway. Adipokines such as adiponectin and leptin induce their metabolic actions on the heart through binding to specific receptors on the cell membrane [47–49]. However, to date the identity of any cardiac receptor of adropin remains to be determined. A recent study suggested adropin's effects on the brain may be mediated through GPR19, a G-protein coupled receptor (GPCR) in neuronal cells [50]. This protein is also expressed in the heart although in lower levels [51]. The more recent study by Thapa et al. [21], proposed GPR19 as the receptor for adropin in H9c2 cells by mediating its metabolic actions on PDH activation through suppression of PDK4 expression in a p44/42 MAPK (ERK1/2)–dependent mechanism. Although our results confirm alterations in this pathway as well as involvement of other pathways affecting insulin sensitivity, further investigation is required to determine the exact receptor/mechanism of action of adropin on the myocardium.

In agreement with our results, a recent paper showed a relative increase in cardiac glucose oxidation in mice on high fat diet treated with adropin injections as compared to other energy substrates [52]. However, in contrast to our direct measurement of actual cardiac metabolic rates over the time course of perfusion and in correlation with functional consequences and changes in mechanical efficiency, that study utilized indirect calculation of steady state enrichment in TCA cycle intermediates following ^{13}C-labbeled glucose venous infusion to estimate the relative contributions of glucose *versus* fatty acid to cardiac acetyl-CoA production [52]. Contrary to our findings in *ex vivo* cardiac function, 3-day adropin treatment did not lead to significant improvement of *in vivo* contractile function [52]. Additionally, the metabolic changes seen with adropin were attributed to alterations in the acetylation status of key metabolic enzymes involved in glucose and fatty acid oxidation rather than PDK4-PDH axis [52], an observation we did not see in our study perhaps due to differences in the treatment protocols and experimental models utilized.

Our data suggests that the signalling pathways mediating adropin's metabolic effects in the heart may involve modulating JNK activity. Sustained JNK activity is known to contribute to endoplasmic reticulum stress [53]. The inhibitory serine phosphorylation of IRS-1 by JNK is known to underline inflammatory- as well as free fatty acid- induced insulin resistance [23,54]. Binding of insulin to its receptor at the extracellular α subunits elicits the intracellular tyrosine autophosphorylation of the β subunits, which enhances the tyrosine kinase activity of the receptor to its downstream adaptor proteins including IRS-1 [55,56]. The phosphorylated tyrosine residues of IRS initiate the activation of downstream kinases and proteins of the insulin signalling pathway including Akt, which in turn phosphorylates and activates AS160 leading to increased GLUT4 translocation to the cell membrane and thus enhance glucose uptake, as well as phosphorylating and inhibiting GSK3β to increase glycogen synthesis and FOXO1 limiting its transcriptional activity on its target genes including PDK4 [24,26,55,57]. As such, attenuation of JNK phosphorylation by adropin is consistent with de-inhibiting IRS-1 mediation of insulin signalling and the observed enhancement of insulin stimulation of glucose oxidation. However, it remains unclear how administration of adropin could lead to decreased JNK phosphorylation in the heart, and whether these effects are mediated downstream of a cardiac adropin receptor. A schematic view of the proposed signalling pathways modulated by adropin is shown in Fig. 8.

5. Conclusions

We demonstrate that adropin has an important role in regulating cardiac energy substrate preference through enhancing cardiac insulin signalling, stimulating glucose oxidation and inhibiting fatty acid oxidation. This action of adropin could potentially provide a promising target in devising therapies for the heart of patients with the metabolic syndrome, obesity, and diabetes, through modulating myocardial energy metabolism. Published clinical research on adropin has been mostly limited so far to observational studies showing correlations between its plasma levels and the consumed diet or several diseases and metabolic parameters such as obesity, cholesterol homeostasis, or the risk of coronary heart disease [30,58–60]. However, the growing body of promising evidence with adropin in pre-clinical studies, including ours, could potentially encourage future clinical trials to explore possible benefits of this compound or its analogues in the treatment of cardiac metabolic conditions such as diabetic cardiomyopathy.

Author contribution

TRA conducted most of the experiments, analyzed their results, and wrote the paper. QGK and AF assisted in immunoblotting. SG was involved in the experimental design of the studies. SR performed acetylation experiments and injections. CSW assisted in perfusion experiments. LZ conducted glucose tolerance tests. GDL coordinated the study and edited the paper with TRA.

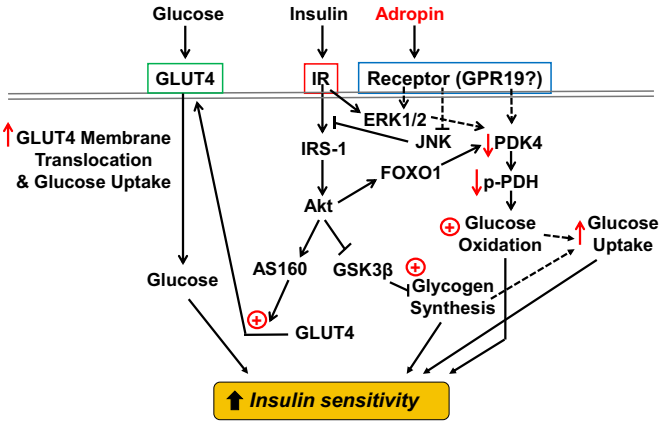

Fig. 8. Proposed signalling pathways that are modulated by adropin resulting in modification of insulin signalling and glucose metabolism. Adropin, possibly through a plasma membrane receptor, such as GPR19 or some other mediator(s), reduces PDK4 protein levels and stimulates ERK1/2 MAPK, which is also known to regulate PDK4 expression, resulting in a decrease in the inhibitory phosphorylation of PDH, the rate limiting glucose oxidation enzyme, leading to its activation and enhancement of glucose oxidation. On the other hand, adropin treatment appears to reduce the phosphorylation of JNK and its activity which otherwise inhibits IRS-1 signalling, thus resulting in an overall stimulation of insulin signalling including downstream targets such as Akt, FOXO1 (that further reduces PDK4 expression), AS160 (increases GLUT4 plasmalemmal translocation and glucose uptake), inhibition of GSK3β (enhances glycogen synthesis). Together, these effects lead to a net enhancement of insulin sensitivity and glucose metabolism and utilization. Akt, protein kinase B; AS160, Akt substrate of 160 kDa; ERK1/2, extracellular signal-regulated kinases 1 & 2; FOXO1, forkhead box protein O1; GPR19, G-protein coupled receptor 19; GSK3β, glycogen synthase kinase 3 beta; IRS-1, insulin receptor substrate 1; JNK, c-Jun NH2-terminal kinase; PDH, pyruvate dehydrogenase; PDK4, pyruvate dehydrogenase kinase 4.

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Declaration of Competing Interest

The authors report no commercial or proprietary interest in any product or concept discussed in this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2019.06.005.

References

[1] Atkinson LL, Fischer MA, Lopaschuk GD. Leptin activates cardiac fatty acid oxidation independent of changes in the AMP-activated protein kinase-acetyl-CoA carboxylase-malonyl-CoA axis. J Biol Chem 2002;277:29424–30.
[2] Lopaschuk GD, Folmes CD, Stanley WC. Cardiac energy metabolism in obesity. Circ Res 2007;101:335–47.
[3] Shioda S, Kageyama H, Takenoya F, Shiba K. Galanin-like peptide: a key player in the homeostatic regulation of feeding and energy metabolism? Int J Obes 2011;35:619.
[4] Al Batran R, Almutairi M, Ussher JR. Glucagon-like peptide-1 receptor mediated control of cardiac energy metabolism. Peptides 2018;100:94–100.
[5] Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011;11:85.
[6] Kumar KG, Trevaskis JL, Lam DD, Sutton GM, Koza RA, Chouljenko VN, et al. Identification of adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism. Cell Metab 2008;8:468–81.
[7] Ganesh-Kumar K, Zhang J, Gao S, Rossi J, McGuinness OP, Halem HH, et al. Adropin deficiency is associated with increased adiposity and insulin resistance. Obesity 2012;20:1394–402.
[8] Gao S, McMillan RP, Jacas J, Zhu Q, Li X, Kumar GK, et al. Regulation of substrate oxidation preferences in muscle by the peptide hormone adropin. Diabetes 2014: DB_140388.
[9] Gao S, McMillan RP, Zhu Q, Lopaschuk GD, Hulver MW, Butler AA. Therapeutic effects of adropin on glucose tolerance and substrate utilization in diet-induced obese mice with insulin resistance. Mol metabolism 2015;4:310–24.
[10] Altamimi TR, Lopaschuk GD. Diabetes and ischemia: similarities in cardiac energy metabolism. Heart Metab 2015;68:4–8.
[11] Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC. Myocardial fatty acid metabolism in health and disease. Physiol Rev 2010;90:207–58.
[12] Buchanan J, Mazumder PK, Hu P, Chakrabarti G, Roberts MW, Yun UJ, et al. Reduced cardiac efficiency and altered substrate metabolism precedes the onset of hyperglycemia and contractile dysfunction in two mouse models of insulin resistance and obesity. Endocrinology 2005;146:5341–9.
[13] Yosaee S, Soltani S, Sekhavati E, Jazayeri S. Adropin-a novel biomarker of heart disease: a systematic review article. Iranian Journal of Public Health, 45; 2016; 1568.
[14] Altamimi TR, Thomas PD, Darwesh AM, Fillmore N, Mahmoud MU, Zhang L, et al. Cytosolic carnitine acetyltransferase as a source of cytosolic acetyl-CoA: a possible mechanism for regulation of cardiac energy metabolism. Biochem J 2018;475:959–76.
[15] Belke DD, Larsen TS, Lopaschuk GD, Severson DL. Glucose and fatty acid metabolism in the isolated working mouse heart. Am J Phys 1999;277:R1210–7.
[16] Larsen TS, Belke DD, Sas R, Giles WR, Severson DL, Lopaschuk GD, et al. The isolated working mouse heart: methodological considerations. Pflugers Archiv: Europ J of Phys 1999;437:979–85.
[17] Altamimi TR, Chowdhury B, Singh KK, Zhang L, Mahmood MU, Pan Y, et al. A novel role of endothelial autophagy as a regulator of myocardial fatty acid oxidation. J Thorac Cardiovasc Surg 2019;157:185–93.
[18] Altamimi TR, Karwi QG, Uddin GM, Fukushima A, Kwong JQ, Molkentin JD, et al. Cardiac-specific deficiency of the mitochondrial calcium uniporter augments fatty acid oxidation and functional reserve. J Mol Cell Cardiol 2019;127:223–31.
[19] Fukushima A, Alrob OA, Zhang L, Wagg CS, Altamimi T, Rawat S, et al. Acetylation and succinylation contribute to maturational alterations in energy metabolism in the newborn heart. Am J Physiol Heart Circ Physiol 2016;311:H347–63.
[20] Ussher JR, Baggio LL, Campbell JE, Mulvihill EE, Kim M, Kabir MG, et al. Inactivation of the cardiomyocyte glucagon-like peptide-1 receptor (GLP-1R) unmasks cardiomyocyte-independent GLP-1R-mediated cardioprotection. Mol metabolism 2014;3:507–17.
[21] Thapa D, Stoner M, Zhang M, Xie B, Manning J, Guimaraes D, et al. Adropin regulates pyruvate dehydrogenase in cardiac cells via a novel GPCR-MAPK-PDK4 signaling pathway. Redox Biol 2018;18:25–32.
[22] Gual P, Le Marchand-Brustel Y, Tanti J-F. Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. Biochimie 2005;87:99–109.
[23] Aguirre V, Uchida T, Yenush L, Davis R, White MF. The c-Jun NH2-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser307. J Biol Chem 2000;275:9047–54.
[24] Gopal K, Saleme B, Al Batran R, Aburasayn H, Eshreif A, Ho KL, et al. FoxO1 regulates myocardial glucose oxidation rates via transcriptional control of pyruvate dehydrogenase kinase 4 expression. Am J Phys Heart Circ Phys 2017;313H479-H90.
[25] Grassian AR, Metallo CM, Coloff JL, Stephanopoulos G, Brugge JS. Erk regulation of pyruvate dehydrogenase flux through PDK4 modulates cell proliferation. Genes Dev 2011;25:1716–33.
[26] Wang Y, Zhou Y, Graves DT. FOXO transcription factors: their clinical significance and regulation. Biomed Res Int 2014;2014.
[27] Reitman ML. FGF21: a missing link in the biology of fasting. Cell Metab 2007;5:405–7.
[28] Scherer PE. Adipose tissue: from lipid storage compartment to endocrine organ. Diabetes 2006;55:1537–45.
[29] Sharma AM, Staels B. Peroxisome proliferator-activated receptor γ and adipose tissue—understanding obesity-related changes in regulation of lipid and glucose metabolism. J Clin Endocrinol Metab 2006;92:386–95.
[30] Butler AA, Tam CS, Stanhope KL, Wolfe BM, Ali MR, O'keeffe M, et al. Low circulating adropin concentrations with obesity and aging correlate with risk factors for metabolic disease and increase after gastric bypass surgery in humans. J Clin Endocrinol Metab 2012;97:3783–91.
[31] Puzio I, Tymicki G, Pawłowska M, Puzio A, Śleboda W. Role of adropin in cardiovascular physiology. Med Weter 2015;71:599–601.
[32] How O-J, Aasum E, Kunnathu S, Severson DL, Myhre ES, Larsen TS. Influence of substrate supply on cardiac efficiency, as measured by pressure-volume analysis in ex vivo mouse hearts. Am J Phys Heart Circ Phys 2005;288H2979-H85.
[33] Jaswal J, Ussher J. Myocardial fatty acid utilization as a determinant of cardiac efficiency and function. Clin Lipidology 2009;4:379–89.
[34] Jaswal JS, Keung W, Wang W, Ussher JR, Lopaschuk GD. Targeting fatty acid and carbohydrate oxidation—a novel therapeutic intervention in the ischemic and failing heart. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research 2011;1813:1333–50.
[35] Horowitz JD, Chirkov YY, Kennedy JA, Sverdlov AL. Modulation of myocardial metabolism: an emerging therapeutic principle. Curr Opin Cardiol 2010;25:329–34.
[36] Zhang L, Jaswal JS, Ussher JR, Sankaralingam S, Wagg C, Zaugg M, et al. Cardiac insulin resistance and decreased mitochondrial energy production precede the development of systolic heart failure following pressure overload hypertrophy. Circulation: Heart Failure. CIRCHEARTFAILURE 2013;112:000228.
[37] Swan JW, Anker SD, Walton C, Godsland IF, Clark AL, Leyva F, et al. Insulin resistance in chronic heart failure: relation to severity and etiology of heart failure. J Am Coll Cardiol 1997;30:527–32.
[38] Ingelsson E, Sundström J, Ärnlöv J, Zethelius B, Lind L. Insulin resistance and risk of congestive heart failure. Jama 2005;294:334–41.
[39] Karwi QG, Zhang L, Wagg CS, Wang W, Ghandi M, Thai D, et al. Targeting the glucagon receptor improves cardiac function and enhances insulin sensitivity following a myocardial infarction. Cardiovascular Diabetology 2019;18:1.
[40] Karwi QG, Zhang L, Altamimi TR, Wagg CS, Patel V, Uddin GM, et al. Weight loss enhances cardiac energy metabolism and function in heart failure associated with obesity. Diabetes, Obesity and Metabolism; 2019.
[41] Karwi QG, Uddin GM, Ho KL, Lopaschuk GD. Loss of metabolic flexibility in the failing heart. Frontiers in cardiovascular medicine 2018;5.
[42] Crewe C, Schafer C, Lee I, Kinter M, Szweda LI. Regulation of pyruvate dehydrogenase kinase 4 in the heart through degradation by the Lon protease in response to mitochondrial substrate availability. J Biol Chem 2017;292:305–12.
[43] Zhabyeyev P, Gandhi M, Mori J, Basu R, Kassiri Z, Clanachan A, et al. Pressure-overload-induced heart failure induces a selective reduction in glucose oxidation at physiological afterload. Cardiovasc Res 2012;97:676–85.
[44] Mori J, Basu R, McLean BA, Das SK, Zhang L, Patel VB, et al. Agonist-induced hypertrophy and diastolic dysfunction is associated with selective reduction in glucose oxidation: a metabolic contribution to heart failure with normal ejection fraction. Circulation: Heart Failure. CIRCHEARTFAILURE 2012;112:966705.
[45] Wiese TJ, Lambeth DO, Ray PD. The intracellular distribution and activities of phosphoenolpyruvate carboxykinase isozymes in various tissues of several mammals and birds. Comparative biochemistry and physiology B, Comparative biochemistry 1991;100:297–302.
[46] Thapa D, Xie B, Manning JR, Zhang M, Stoner MW, Huckestein BR, et al. Adropin reduces blood glucose levels in mice by limiting hepatic glucose production. Physiol Rep 2019;7:e14043.
[47] Iwabu M, Okada-Iwabu M, Yamauchi T, Kadowaki T. Adiponectin/adiponectin receptor in disease and aging. NPJ aging and mechanisms of disease 2015;1:15013.
[48] Hall ME, Harmancey R, Stec DE. Lean heart: role of leptin in cardiac hypertrophy and metabolism. World J Cardiol 2015;7:511.
[49] Kielar D, Clark J, Ciechanowicz A, Kurzawski G, Sulikowski T, Naruszewicz M. Leptin receptor isoforms expressed in human adipose tissue. Metabolism 1998;47:844–7.
[50] Stein LM, Yosten GL, Samson WK. Adropin acts in brain to inhibit water drinking: potential interaction with the orphan G protein-coupled receptor, GPR19. Am J Phys Regul Integr Comp Phys 2016;310R476-R80.
[51] Hoffmeister-Ullerich SA, Süsens U, Schaller HC. The orphan G-protein-coupled receptor GPR19 is expressed predominantly in neuronal cells during mouse embryogenesis. Cell Tissue Res 2004;318:459–63.
[52] Thapa D, Xie B, Zhang M, Stoner MW, Manning JR, Huckestein BR, et al. Adropin treatment restores cardiac glucose oxidation in pre-diabetic obese mice. J Mol Cell Cardiol 2019;129:174–8.
[53] Win S, Than T, Fernandez-Checa J, Kaplowitz N. JNK interaction with Sab mediates ER stress induced inhibition of mitochondrial respiration and cell death. Cell Death Dis 2015;5:e989.
[54] Hirosumi J, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, et al. A central role for JNK in obesity and insulin resistance. Nature 2002;420:333.

[55] Chang L, Chiang S-H, Saltiel AR. Insulin signaling and the regulation of glucose transport. Mol Med 2004;10:65.
[56] Zhang L, Keung W, Samokhvalov V, Wang W, Lopaschuk GD. Role of fatty acid uptake and fatty acid β-oxidation in mediating insulin resistance in heart and skeletal muscle. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids 2010;1801:1–22.
[57] Fischer Y, Thomas J, Sevilla L, Muñoz P, Becker C, Holman G, et al. Insulin-induced recruitment of glucose transporter 4 (GLUT4) and GLUT1 in isolated rat cardiac myocytes. Evidence of the existence of different intracellular glut4 vesicle populations. J Biol Chem 1997;272:7085–92.
[58] Ghoshal S, Stevens JR, Billon C, Girardet C, Sitaula S, Leon AS, et al. Adropin: an endocrine link between the biological clock and cholesterol homeostasis. Molecular metabolism 2018;8:51–64.
[59] Stevens JR, Kearney ML, St-Onge MP, Stanhope KL, Havel PJ, Kanaley JA, et al. Inverse association between carbohydrate consumption and plasma adropin concentrations in humans. Obesity 2016;24:1731–40.
[60] Aydin S, Eren MN, Yilmaz M, Yardim M, Aydin S, Kalayci M, et al. Adropin as a potential marker of enzyme-positive acute coronary syndrome. Cardiovasc J Afr 2017;28:40.