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Acute Adiponectin Delivery is Cardioprotective in the Aged Female Rat Heart

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Abstract

The aged, post-menopausal female heart is characterized by reduced ischemic tolerance, and few therapies currently exist to limit ischemic damage. Adiponectin (APN), a cytokine produced in adipose tissue, limits infarct size and improves functional recovery following ischemia reperfusion (I/R) injury in adult hearts.

Aim—To extend these previous studies and determine the cardio protective efficacy of APN treatment in aged female rats.

Methods—hearts were isolated from adult (6–7 mo; n=10), aged (23 mo; n=14), and aged ovariectomized (OVX; n=10) female rats and subjected to I/R injury. Upon ischemia, hearts were infused with 9µg of APN or vehicle. Adiponectin receptor1 (AdipoR1), AdipoR2, and adenosine-monophosphate dependent kinase (AMPK) were assessed by western blotting, tumor necrosis factor (TNFα) and nicotinamide adenine dinucleotide phosphateoxidase (NOX2/NOX4) levels by real time PCR. Non-reducing western blotting for adiponectin multimers in visceral adipose was also performed.

Results—APN infusion successfully improved post-ischemic left ventricular developed pressure (~10–15%) and attenuated the rise in end diastolic pressure (EDP) in all groups (p<0.05). With I/R injury, phospho-AMPK increased in all groups with additive effects of APN on increasing phospho-AMPK abundance in aged ovary-intact only (p<0.001). Age-associated increases in preischemic TNFα mRNA were unaffected by APN, while NOX 2 mRNA levels were attenuated

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J. Craig Hunter: I declare that I participated in the data collection, data analysis, editing a draft of this manuscript, and that I have seen and approved the final version. I have no conflicts of interest.

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Veronica Lopez: I declare that I participated in the data collection and data analysis, and that I have seen and approved the final version. I have no conflicts of interest.

Donna H. Korzick: I declare that my contributions involved conception and design of this research project, interpretation of data, editing several drafts of this manuscript, and as corresponding author I have approved the final version. I have no conflicts of interest.

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by APN in adult and aged OVX. An age-associated decrease in cardiac AdipoR2 was observed in conjunction with elevated high molecular weight APN in adipose.

Conclusions—Our data suggest that APN may be a relevant therapy for protecting the aging female heart, albeit through divergent mechanisms which are likely influenced by age-associated estrogen availability.

Keywords

ischemia; reperfusion; menopause; TNF α ; AMPK; AdipoR

INTRODUCTION

Adiponectin (APN) is an anti-diabetic, anti-atherogenic, anti-inflammatory, and cardio protective adipokine that circulates at concentrations of 3–30 $\mu\text{g/mL}$ ^{1–5}. Hypoadiponectinemia ($< 4 \mu\text{g/mL}$) is correlated with insulin resistance, glucose intolerance, type II diabetes, obesity-linked inflammation, and increased risk for metabolic syndrome and coronary heart disease (CHD) ^{1–8}. Interestingly, APN treatment improves cardiovascular health not only by reducing co-morbidities for CHD, but also by directly protecting the ischemic myocardium. Following ischemic insult, bolus delivery of APN reduces infarct size and limits the decline in functional recovery in both rodent and porcine models ^{9–14}.

APN has been reported to protect the myocardium following ischemic insult via three independent mechanisms ^{13–15}. During ischemia, APN treatment promotes cyclooxygenase 2 (COX2)–mediated suppression of tumor necrosis factor (TNF) α , thereby reducing inflammation ¹³. APN also inhibits peroxynitrite formation via inhibition of the superoxide producing subunit of nicotinamide adenine dinucleotide phosphate (NADPH), gp91^{phox} ^{15, 16} (NOX2). Both TNF α and NOX2 are known to be negatively regulated by estrogen ¹⁵. Additionally, APN also reduces apoptotic cell death via an adenosine monophosphate activated protein kinase (AMPK)–dependent mechanism ^{13, 17}. AMPK is of particular interest given its dual role in promoting cardioprotection and damage following ischemic injury. When activated by phosphorylation on site Thr172, AMPK promotes both beneficial glucose utilization and detrimental enhancement of fatty acid utilization ^{18–21}. Currently, the exact mechanisms involving the interplay of AMPK-directed fuel utilization and APN/AMPK-dependent suppression of apoptosis are incompletely understood. However, aging is associated with reduced basal AMPK phosphorylation in cardiac muscle and a reduced capacity for APN-mediated AMPK activation ^{22, 23}. This indicates the aged heart may demonstrate a limited ability to respond to cardioprotective APN treatment during an ischemic insult.

Indeed, diabetic rodent models display APN-resistance, and APN treatment is less efficacious in attenuating ischemic injury in these models ^{14, 24}. In fact three-times the APN dose is needed to protect diabetic mouse hearts from ischemic injury ¹⁴. Attenuated APN efficacy has also been linked to both reduced AMPK activation and endogenous APN concentrations as well as compensatory increases in cardiac adiponectin receptor 1 (AdipoR1) protein levels ¹⁰. Thus, the relevance of APN as a cardioprotective agent for the

aging heart is questionable given the prevalence of metabolic disease co-morbidities, diabetes and obesity.

Aging women represent a unique population which demonstrates increased adiposity, reduced ischemic tolerance, and progression toward metabolic dysregulation following the onset of estrogen deficiency at the menopausal transition. Testing APN efficacy in a model of aging and estrogen deficiency will help determine if APN could be a relevant therapy to treat ischemic heart disease for aging post-menopausal women^{25, 26}. As such, the primary focus of this investigation was to determine the efficacy of acute APN delivery in protecting aged female Fisher 344 (F344) rats subjected to cardiac I/R injury. While aging F344 rats are not diabetic, they are characterized as insulin resistant and demonstrate increased adiposity, reduced circulating APN concentrations, and reduced ischemic tolerance similar to adult diabetic animal models^{14, 27, 28}. To elucidate potential effects of age-associated estrogen deficiency on downstream APN signaling, we also characterized cardiac AdipoR subtype abundance and AMPK phosphorylation status, as well as pre and post-ischemic mRNA levels for TNF α , NOX2 and NOX4.

MATERIALS AND METHODS

Animal Care

All animal experimentation was conducted with approval from the Institutional Animal Care and Use Committee of the Pennsylvania State University and in accordance with provisions of the Declaration of Helsinki (Seoul 2008, <http://www.wma.net/en/30publications/10policies/b3/index.html>). Certified specific pathogen-free female adult and aged F344 rats were obtained from the National Institute on Aging/Taconic (Hudson, NY). All rats were either 6–7 mo (adult, n=10) or 23 mo (aged, n=14) at the time of study. Rats were singly housed in filter-top cages containing corn-cob bedding and provided with a nylabone or wood block for enrichment. Rats were maintained on a 12 h light/dark cycle. Water and chow were supplied *ad libitum* and chow consisted of a standard laboratory rodent diet, Lab Diet 5001 (PMI Nutrition International, St. Louis, MO) with a caloric breakdown of 28.5% protein, 13.5% fat, and 58% carbohydrate.

Ovariectomy

Because aged F344 female rats demonstrate variable anestrus, ovariectomy (OVX) is an effective method to yield a consistent level of estrogen deficiency. Thus, an additional group of aged rats (n = 10) were subject to OVX by the animal supplier, Taconic at 22 mo. We have consistently observed significant reductions in circulating estrogen concentrations in aged vs adult ovary intact (~14 pg/mL vs ~28 pg/mL), with further reductions in aged OVX (~ 8 pg/mL)^{29, 30}. Following surgery, animals were allowed to recover for four weeks prior to experimental use. Uterine weight was used to confirm estrogen deficiency as previously validated in our laboratory^{30, 31}.

Isolated Heart Protocol

Rats were anesthetized with pentobarbital (40 mg/kg body weight; intraperitoneal), and hearts were excised via midline thoracotomy. Isolated hearts were secured to a modified

Langendorff perfusion apparatus via the aorta, perfused with a modified Krebs-Henseleit buffer (~1.25 mM Ca^{2+} final) at a perfusion pressure of 85 mmHg, and hearts were paced at 260 beats/min. Left ventricular developed pressure (LVDP), and positive and negative developed pressure with respect to time ($\pm$ $\text{dP/dt}_{\text{max}}$; indices of contractility and relaxation, respectively), were assessed by a pressure transducer attached to a water-filled latex balloon inserted into the LV. End diastolic pressure (EDP) was adjusted to 5–6 mmHg according to established procedures in our laboratory^{30, 31}. After 30 min of equilibration, hearts were subjected to 47 min global isothermic (37°C) ischemia as described previously³¹. Pacing was re-initiated 1 min 30 sec after the start of reperfusion and hearts were reperused for 60 min as previously described³¹. LV tissue was isolated, weighed, halved, and snap frozen in liquid N_2 and subsequently stored at -80°C until biochemical analysis. Rats with pre-ischemic LVDP below 130 mmHg, unstable pre-ischemic EDP (> 6 mmHg), or notable pathology/weight loss were excluded.

APN Treatment Study Design

Adult, aged, and aged OVX rats were evenly divided and randomly assigned to receive an infusion of either 9 μg of APN (Biovondor) or vehicle (Krebs-Henseleit perfusion buffer) delivered in 2 mL for 1 min upon initiation of global ischemia (see Figure 1). The APN dose and timing of delivery was chosen based on efficacy demonstrated in past studies with male Sprague-Dawley rats and was delivered via a side arm perfusion pump connected to the Langendorff apparatus¹². To account for the influence of circadian rhythm on cardiac function, all experiments were conducted between 9:00 am and 2:00 pm on a given day.

Tissue Homogenization

LV tissue was minced and homogenized by glass-glass grinding in buffer containing : 250 mM sucrose; 10 mM Tris-HCl, pH 7.4; 1 mM EDTA, pH 7; 1 mM ortho-vanadate; 1 mM NaF; 0.3 mM PMSF; 5 $\mu\text{g}/\text{ml}$ each of leupeptin and aprotinin; 0.5 $\mu\text{g}/\text{ml}$ pepstatin A, and 1% Triton-X100. LV tissue was then subjected to a 100,000 X g centrifugation for 60 min. The supernatant was assessed for total protein concentration using the Bradford method³².

Western Blotting and Adiponectin ELISA

Protein lysates were subjected to separation using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to polyvinylidene fluoride membranes and blocked in 5% non-fat dry milk for 2 hours at room temperature as described, previously^{30, 31}. Membranes were probed overnight at 4°C with primary antibodies at a dilution of 1:1000 for AdipoR1 (42 kDa, AdipoR1-2; Alpha Diagnostics), 1:2000 for AdipoR2 (42 kDa, AdipoR2-1; Alpha Diagnostics), 1:500 for phosphorylated AMPK (phospho-AMPK, Thr-172; 62 kDa; 2535; Cell Signaling), and 1:1000 for AMPK (63 kDa, 07-350; Millipore). Membranes were then incubated with HRP-linked anti-rabbit secondary antibody at a dilution of 1:20,000 for 1 h at 28°C and visualized using ECL (GE Healthcare). Densitometry was performed using Scion Image (NIH). To correct for potential protein loading errors all membranes were stained with Sypro Ruby blot stain (Invitrogen) and densitometry was performed as previously described^{30, 31}. Rat serum was assessed for total APN concentration utilizing a kit (EZRADP-62K; Millipore) per manufacturer instructions.

Non-Reducing Western Blotting for Adiponectin Multimers

Visceral adipose was collected immediately following heart extraction. Perimetrial fat pads were isolated, rinsed in saline, blotted dry, and weighed. Protein lysates were subject to separation under non-reducing conditions. Electrophoresis was performed on a 4–20% gradient gel (BioRad) without the β -mercaptoethanol in the sample buffer, and without SDS in the sample and electrophoresis buffers. Samples were transferred and blotted for APN as indicated in standard western protocol.

Real Time PCR

RNA was obtained from frozen LV tissue through acid guanidiniumthiocyanate-phenol-chloroform extraction with Tri Reagent (Sigma Chemical Co., St. Louis, MO) as described by Chomczynski and Sacchi³³. Extracted RNA was reverse transcribed to cDNA and subjected to quantitative real-time-PCR using the iQ SYBR Green Supermix according to the manufacturer's protocol (Biorad Single Color Real-time PCR Detection System (MyIQ Optics Module)). Gene expression levels of TNF α , NOX1, NOX2 and NOX4 were analyzed. Primers were manually designed using Primer3 (<http://bioinfo.ut.ee/primer3-0.4.0/>). The PCR cycling parameters were as follows: 95 °C for 3 min, and 40 cycles of 95 °C for 15 s, 60 °C for 30 sec and 72 °C for 30 sec. Each sample was analyzed in triplicate and normalized to the housekeeping gene, cyclophilin, using the following equation: $Ct_{ER\beta} = Ct_{ER\beta} - Ct_{cyclophilin}$ where Ct is the linear part of the curve. The fold change in mRNA expression was calculated using the following equation: $2^{(-\Delta Ct)}$ where $Ct = \text{mean } Ct \text{ of TNF}\alpha, \text{ NOX2 or NOX4 in F344 heart homogenates.}$ NOX1 mRNA levels were below the limits of detection in our hands.

Statistical Analysis

All data are presented as means $\pm$ standard error (SE) and analyzed using the SAS general linearized models (GLM) procedure. A one-way ANOVA was used to analyze morphological characteristics, circulating APN concentrations and AdipoR protein level data for adult, aged, and aged OVX animals, respectively. A two-way ANOVA (group X drug) was used to analyze all functional and mRNA data for adult, aged, and aged OVX animals with and without APN treatment. A three way ANOVA (group X drug X I/R) was used to analyze phospho-AMPK, and AMPK protein levels for adult, aged, and aged OVX animals subject to control perfusion, or I/R with or without drug. The Tukey test was used for all *post hoc* analysis. An α -level of $p < 0.05$ was considered statistically significant.

RESULTS

Baseline Morphological and Functional Characteristics

Baseline characteristics did not differ statistically between APN and vehicle-treated rats, and were pooled for analysis (Table 1). Body, LV, and gonadal adipose depot weights were significantly greater in aged and aged OVX vs adult control rats ($p < 0.001$); there were no differences between aged and aged OVX rats. As expected, uterine weight decreased significantly with OVX ($p < 0.001$). Pre-ischemic LVDP, but not $\pm$ dP/dt, was significantly reduced in aged and aged OVX vs adult ($p < 0.05$). Finally, and as we have

observed previously³⁴, circulating APN was significantly reduced in aged vs adult (12.5 ± 1.1 vs 17.6 ± 0.9 $\mu\text{g/mL}$; $p < 0.01$), however no differences were observed between adult and aged OVX (18.01 ± 1.3 $\mu\text{g/mL}$). High molecular weight (HWW) APN was significantly increased in aged and aged OVX vs adult, while increases in medium molecular weight (MMW) and low molecular weight (LMW) APN were only observed in Aged OVX (Figure 1; $p < 0.05$).

Adiponectin Infusion, Post-Ischemic LV Function

Aged and aged OVX rats demonstrated reduced functional recovery following 47 min ischemia vs adult controls (Figure 2; $p < 0.001$). However, APN infusion upon ischemia was successful in improving functional recovery in adult, aged, and aged OVX ($p < 0.001$). Specifically, LVDP was improved in all groups with APN infusion by 10–20% (Figure 2, Panels A-C). Similar to LVDP, decrements in $\pm dP/dt_{\text{max}}$, were also attenuated following ischemia in all groups ($p < 0.001$; Figure 2, Panels D-I) with APN treatment. As expected, post-ischemic EDP was significantly increased (~100 fold) in vehicle-treated rats but a significant reduction of ~15–35 mmHg was observed in all APN- treated groups ($p < 0.05$; Figure 3).

AMPK Phosphorylation and AdipoR Protein Levels

Interestingly, while phospho-AMPK levels increased in response to the I/R protocol in all groups ($p < 0.001$; Figure 4B), no additional increase in phospho-AMPK was observed with APN infusion in any group. In fact, APN treatment in adult rats resulted in a significant decrease in phospho-AMPK relative to those treated with vehicle ($p < 0.05$). When corrected for total AMPK protein levels, a significant increase in the ratio of phospho-AMPK to total AMPK following APN emerged for the aged group only (Figure 4 Panel D). While AdipoR1 protein abundance isolated from LV tissue was not significantly different across groups (Figure 5 Panel A), AdipoR2 levels were significantly decreased in aged vs adult hearts ($p < 0.05$; Figure 5 Panel B).

TNF α , NOX2 and NOX4 mRNA Levels

In the absence of ischemia, TNF α and NOX2 mRNA levels were significantly greater in aged vs adult (Figure 6A and 6C), while group differences in NOX4 were not observed (data not shown). TNF α mRNA levels measured following I/R injury (60 min) remained significantly increased in aged and aged OVX (Figure 6B) relative to adult, with no apparent effect of APN pretreatment. NOX2 mRNA levels were significantly reduced by APN pretreatment in hearts isolated from adult and aged OVX rats following I/R injury, while levels in the aged group were increased. A closer examination of the individual responses in the aged group revealed APN responders ($n=4$) and non-responders ($n=3$). Group effects on post-ischemic NOX4 levels were not observed (data not shown).

DISCUSSION

APN has direct cardioprotective effects in response to I/R injury in adult male mice, rats, and pigs^{9, 11–13, 15}. However, no previous studies have addressed APN-mediated cardioprotection with advancing age or with female sex. Here, we demonstrate for the first

time that APN is effective in improving LV functional recovery following ischemia in adult and age female rat hearts. Toward a mechanism of this protection, we found divergent group effects on several known effectors of APN-mediated cardioprotection. That AMPK phosphorylation, TNF α and NOX levels are differentially regulated following I/R injury with APN treatment in adult vs aged females hearts, provides novel mechanistic insights for APN efficacy in the female rat heart.

Similar to findings in adult male Sprague-Dawley rats ¹², post-ischemic LVDP and $\pm$ dP/dt_{max} were significantly improved with a single 9 μ g dose of APN delivered upon ischemia in adult, aged, and aged OVX female rat hearts. APN also attenuated the post-ischemic rise in EDP in all experimental groups. Accordingly, our findings extend previous knowledge demonstrating APN-mediated cardioprotection in adult males to the adult, aged, and aged OVX female heart. While infarct size analysis was not performed, improved post-ischemic mechanical function supports the assertion that APN may be a relevant cardio protective therapy for myocardial infarction in aged post-menopausal women and is worthy of further investigation.

Notably, prior experimental evidence suggest that endogenous circulating APN concentrations are important in predicting ischemic tolerance ^{10, 14}. We have previously shown that while circulating APN concentrations are reduced in aged female rats with ovaries intact, APN is significantly increased following OVX in aged rats ³⁴. As such, the interaction of age and OVX results in similar circulating APN concentration as adult females, and results were replicated in the current study ³⁴. Here, we also assessed APN processing. HMW APN in adipose tissue was found to be significantly elevated in aged, which may provide a mechanism, at least in part, for observed reductions in circulating APN. However, that circulating APN is higher in aged OVX in spite of increased adipocyte HMW APN, presents a more complicated intra-adipocyte APN regulatory processing scenario which may be more related to observed increased levels of LMW and MMW. Alternatively, increased levels of circulating APN in aged OVX may represent a compensatory adaptation triggered by a threshold level reduction in circulating estradiol. That we saw APN-mediated protection in all experimental groups suggests adult and aged OVX animals (which have the highest endogenous APN concentrations) may have a lower threshold dose requirement for APN-mediated protection. Future studies addressing therapeutic dosing are indicated to address this important issue.

AdipoR1 and AdipoR2 protein levels were also characterized in our model to focus on potential mechanism(s) of APN action in a setting of age-associated estrogen deficiency. It has been suggested by Shibata et. al³⁵ that APN accumulates in the myocardium following I/R injury as a means of activating protective signaling. Accordingly, we assessed AdipoR1 and AdipoR2 levels to determine if and which APN signaling cascade(s) may be mediating the observed cardioprotection. We observed a small but significant age-related decrease in AdipoR2. While a role for AdipoR2 in I/R injury has yet to be identified ³⁶, we have previously shown similar age-related reductions in AdipoR2 protein levels in adipose tissue ³⁴. It is unclear at this time if the change observed here is indicative of a novel adaptation of the aged female heart or if reduced AdipoR2 is simply an attribute of the F344 female rat model.

Toward elucidating a mechanism for the observed APN-mediated protection, we assessed the phosphorylation status of the downstream target AMPK. Phospho-AMPK increased in response to ischemic injury in adult and aged rats which is in accordance with previous reports^{10, 12–14}. However, additive increases in phospho-AMPK were observed with APN treatment in aged-ovary intact rats only. The variable response in adult and aged females is curious given that *in vivo* coronary artery ligation studies report sustained phospho-AMPK levels up to 24 and 48 h of reperfusion following APN treatment^{10, 13, 14}. A possible explanation for the divergent results observed in the current study vs prior *in vivo* models includes the effect of a fatty acid-free perfusion buffer for isolated heart studies as well as timing of sample collection. In isolated working hearts, treatment with APN under normoxic conditions in the presence of fatty acids does result in a 40% increase in phospho-AMPK³⁶. Sex and/or rodent strain differences may also account for divergent AMPK responses associated with APN treatment in our model. Sufficiency of the phospho-AMPK response in combination with APN induced reductions in NOX2 may explain the protection in adult. Regardless of the cause, however, our data suggest that AMPK activation is not likely a critical mediator of APN-mediated cardioprotection in the F344 female rat heart. The lack of increase in phospho-AMPK in aged OVX rats taken with significant improvement in functional recovery in all groups further supports this notion. Alternative mechanisms of protection include a COX2-mediated suppression of TNF α induced inflammation^{13, 37} or attenuation of cytotoxic nitrate production¹⁵. Tissue analysis of TNF α mRNA in post-ischemic LV revealed age-related increases which were unaffected by APN treatment. Attempts to assess cardiac-specific TNF α accumulation following ischemic injury in perfusion effluent were unsuccessful due to lack of a sufficiently sensitive commercially available assay for this medium. Because APN also inhibits peroxynitrite formation via inhibition of the superoxide producing subunit of NADPH, gp91^{phox} in the ischemic myocardium, we measured NOX2 and NOX4 levels. We observed a significant attenuation of NOX2 levels in adult and aged OVX, while APN effects in the aged group were divergent. Specifically, there was a pattern of responders and non-responders in the aged group, which may have been influenced by variable estradiol levels known to occur in this group^{29, 30, 38}. Limitations of post-ischemic mRNA assessment include possible variability introduced by ischemic injury that is variable between groups. Nevertheless, a pattern of protection through a NOX2 mechanism is consistent with previous studies in adult animals, which we now extend to the aged, estrogen deficient rat heart.

In conclusion, we have demonstrated that the aged female rat heart is responsive to APN treatment following ischemic insult. Additionally, we observed divergent changes in phospho-AMPK and NOX2 activation in response to ischemic injury toward a mechanism of APN-mediated cardio protection in adult, aged and aged OVX female rats. This discordance suggests mechanisms of APN action may differ in estrogen replete and deficient animals. Further investigation of alternative APN downstream targets will prove useful in elucidating relevant therapy for aged post-menopausal women.

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Figure 1.

Representative blots and adipose protein levels for adiponectin (APN) multimers processed under non-reducing conditions. High molecular weight (HWM) APN (Panel A), medium molecular weight (MMW) APN (Panel B), low molecular weight (LWM) APN (Panel C), monomeric (Panel D); representative non-reducing blot (Panel E). * denotes significantly different from adult intact, † denotes significantly different from aged; ($p < 0.05$; $n=5-6$ /group). Values are means $\pm$ SEM; data is presented relative to adult ovary intact.

Figure 2.

Adiponectin delivery improves post-ischemic left ventricular developed pressure (LVDP) and change in pressure with respect to time ($+/-dP/dt_{max}$) in the female rat heart. Recovery of LVDP following a 47 min ischemia in adult (Panel A), aged (Panel B), aged OVX (Panel C) female rats. Recovery of $+/-dP/dt_{max}$ following a 47 min ischemia in adult (Panel D and G), aged (Panel E and H), aged OVX (Panel F and I) female rats.* denotes different from adult; ‡ denotes main effect of drug; $p < 0.05$; $n=3-6$ /group.

Figure 3.

Adiponectin delivery improves post-ischemic end diastolic pressure (EDP) in female rat heart. Recovery of EDP following a 47 min ischemia in adult (Panel A), aged (Panel B), aged OVX (Panel C) female rats. ‡ denotes main effect of drug; $p < 0.05$; $n=3-6$ /group.

Figure 4.

Adenosine monophosphate-dependent protein kinase (AMPK), activated AMPK (phospho-AMPK), and the phospho-AMPK/AMPK ratio in isolated hearts perfused for 30 min (Perfused Control), or subjected to ischemia/reperfusion (I/R; 47/60 min) with vehicle, or adiponectin (APN) infusion (9 μ g) delivery upon ischemia. Representative blots for phospho-AMPK and AMPK (Panel A), protein levels for phospho-AMPK (Panel B), protein levels for AMPK (Panel C) and the ratio of phospho-AMPK/AMPK (Panel D). * denotes different from adult perfused control, † denotes different from aged perfused control; § denotes

different from adult I/R; $p < 0.05$; $n=3-5/\text{group}$). Values are means $\pm$ SEM; data is presented relative to adult perfused control and corrected with Sypro Ruby Blot Stain.

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Figure 5.

Adiponectin Receptor 1 (AdipoR) 1 and AdipoR2 in isolated hearts. Representative blots and protein levels AdipoR1 (Panel A), and AdipoR2 (Panel B). Values are means $\pm$ SEM; data is presented relative to adult and corrected with Sypro Ruby Blot Stain; * denotes different from adult group; $p < 0.05$; $n=3-5$ /group.

Figure 6.

mRNA levels for Tumor Necrosis Factor α (TNF α) and nicotinamide adenine dinucleotide phosphate (NADPH) (gp91phox; NOX2) prior to (Panels A and C) and following ischemia/reperfusion injury (Panels B and D) in the female rat heart. Each sample was normalized to the housekeeping gene, cyclophilin. * denotes different from adult group; $p < 0.05$.

Table 1

Baseline morphological and functional characteristics.

Characteristic	Adult	Aged	Aged OVX
N	11	9	7
Body Weight g	208.00 ± 1.00	296.00 ± 2.00 *	299.00 ± 3.00 *
LV weight, mg	581.60 ± 5.80	779.60 ± 6.10 *	761.00 ± 8.70 *
LV/body weight, mg/g	2.80 ± 0.01	2.60 ± 0.01	2.50 ± 0.01
Uterine Weight, g	0.67 ± 0.02	0.67 ± 0.01	0.34 ± 0.01 †
Gonadal Adipose Weight, g	3.41 ± 0.16	7.30 ± 0.27 *	7.17 ± 0.11 *
EDP, mmHg	5.50 ± 0.10	5.80 ± 0.10	5.90 ± 0.10
LVDP, mmHg	145.70 ± 1.30	134.90 ± 0.50 *	139.10 ± 1.40 *
+dP/dt, mmHg/sec	3907.00 ± 66.00	3865.00 ± 33.00	3757.00 ± 47.00
-dP/dt, mmHg/sec	2541.00 ± 40.00	2328.00 ± 21.00	2543.00 ± 37.00

OVX, ovariectomized; LV left ventricle; EDP end diastolic pressure; LVDP left ventricular developed pressure; dP/dt first derivative of LVDP.

* denotes different from adult;

† denotes different from aged; p<0.05.