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Heart Failure with Preserved Ejection Fraction Induces Beiging in Adipose Tissue

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Abstract

Background—Despite the increasing prevalence of heart failure with preserved ejection fraction (HFpEF) in humans, there are no evidence-based therapies for HFpEF. Clinical studies suggest a relationship between obesity-associated dysfunctional adipose tissue (AT) and HFpEF. However an apparent obesity paradox exists in some HF populations with a higher BMI. We sought to determine if HFpEF exerted effects on AT and investigated the involved mechanisms.

Methods and Results—Mice underwent *d*-aldosterone infusion, uninephrectomy, and were given 1% saline for 4 weeks. HFpEF mice developed hypertension, left ventricular hypertrophy, diastolic dysfunction and had higher myocardial natriuretic peptide expression. Although body weights were similar in HFpEF and sham-operated mice, white AT (WAT) was significantly smaller in HFpEF than Sham (epididymal AT: 7.59 vs. 10.67 mg/g; inguinal AT: 6.34 vs. 8.38 mg/g). These changes were associated with smaller adipocyte size and increased beiging markers (ucp-1, cidea and eva) in WAT. Similar findings were seen in HFpEF induced by TAC. Increased activation of natriuretic peptide signaling was seen in WAT of HFpEF mice. The ratio of the signaling receptor, npra, to the clearance receptor, nprc, was increased as was p38 MAPK activation. However, HFpEF mice failed to regulate body temperature during cold temperature exposure. In HFpEF, despite a larger brown AT mass (BAT; 5.96 vs. 4.50 mg/g), BAT showed reduced activity with decreased ucp1, cidea and eva expression, and decreased expression of lipolytic enzymes (hsl, lpl and fabp4) vs. Sham.

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Disclosures

None.

Conclusions—These findings show that HFpEF is associated with beiging in WAT and with dysfunctional BAT.

Keywords

heart failure with preserved ejection fraction; adipose tissue; beiging; natriuretic peptides

Heart failure (HF) with preserved ejection fraction (HFpEF) is a clinical syndrome characterized by lung congestion, increased left ventricular (LV) filling pressure and diastolic dysfunction (DD), with preservation of LV ejection fraction (LVEF)^{1, 2}. In contrast to HF with reduced ejection fraction (HFrEF), there are no evidence-based therapies for HFpEF. Randomized clinical trials, to date, in patients with HFpEF, failed to show any improvement in morbidity or mortality^{3–6}. Management is limited to the treatment of symptoms and comorbidities. Uncertainties surround the mechanism and pathophysiology of HFpEF; with HFpEF patients having more comorbidities than those with HFrEF, e.g., arterial hypertension (HTN), metabolic abnormalities such as diabetes, hyperlipidemia and obesity^{2, 7–10}.

Obesity is significantly associated with the future development of HF despite adjusting for established risk factors¹¹. In addition to other known risk factors, such as HTN and coronary artery disease, obesity is highly prevalent in population-based studies and registries of HFpEF^{2, 12}. Similarly, dysfunction of the vasculature, kidney, skeletal muscle, and adipose tissue (AT) are important contributors to the HFpEF phenotype^{13, 14}. The role of obesity and adipose derived adipokines has provided some insights into the onset and evolution of HFpEF¹⁵. Recently we showed that factors released by AT, such as adiponectin, may regulate the pathological heart or interact with neurohormones in HFpEF¹⁶. Similarly cardiac cells release regulatory peptides, called cardiokines, in response to changes in the cardiac environment¹⁷. These cardiokines have physiological and pathological roles in organs distal from the heart, such as muscle, fat and liver; where cell death, growth, fibrosis and remodeling are regulated^{18, 19}. However, much less is known about their role in mediating metabolic crosstalk between the heart and peripheral tissues^{19, 20}. In addition to natriuresis, diuresis, and vasodilation, natriuretic peptides released from the heart enhance lipolysis and lipid mobilization^{21, 22}. Moreover, it has also been described that these natriuretic peptides induce changes in white AT (WAT) depots promoting the development of brown AT (BAT) characteristics (such as increased thermogenesis and increase energy expenditure), a phenomenon termed “browning” or “beiging”²³. These findings provide further evidence of communication between the heart and peripheral tissues. However, it is unknown if cardiokines and the heart may regulate the AT phenotype and biology in non-obese HFpEF. We thus sought to investigate whether AT function is regulated in HFpEF in non-obese mice as this may provide mechanistic insight into the relationship between HFpEF and obesity and similarly set the stage for the development of novel treatments for HFpEF.

Methods

A detailed methods section is available in the Data Supplement.

Experimental model

The Institutional Animal Care and Use Committee at Boston University School of Medicine approved all study procedures related to the handling and surgery of the mice. There were 2 groups of mice experiments.

Eight-week C57BL/6J old mice were anesthetized with 80–100 mg/kg ketamine and 5–10 mg/kg xylazine intraperitoneally. Mice (20–25 g) underwent uninephrectomy and then received either a continuous infusion of saline (Sham) or *d*-aldosterone (0.30 µg/h, Sigma-Aldrich, St. Louis, MO) (HFpEF) for 4 weeks via osmotic minipumps (Alzet, Durect, Cupertino, CA). The animals were sacrificed after 4 weeks at which time there was a HFpEF phenotype^{16, 24}.

Another group of WT mice underwent C57BL/6J mice underwent transverse aortic constriction (TAC). Briefly, mice were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally). The chest was opened, and following blunt dissection through the intercostal muscles, the thoracic aorta was identified. Silk suture (7–0) was placed around the transverse aorta and tied around a 26-gauge blunt needle, which was subsequently removed. Sham-operated mice underwent a similar surgical procedure without aortic constriction. After 14 days, cardiac structure and function were determined by echocardiography. Mice were then sacrificed, the hearts were weighed and white adipose depots were collected.

Physiological measurement

Heart rate (HR) and blood pressure (BP) were measured weekly using a noninvasive tail-cuff blood pressure analyzer, BP-2000 Blood Pressure Analysis System (Visitech Systems, Inc., Apex, NC)^{16, 24}. Transthoracic echocardiography was performed at the end of 4 weeks in chronic aldosterone and after 14 days in TAC using the Vevo 770 High-Resolution In Vivo Micro-Imaging System and a Real-Time Micro Visualization 707B Scanhead (VisualSonic Inc., Toronto, Ontario, Canada) as previously described¹⁶. See data supplement for further details. The ratio of wet to dry lung weight ratio was used as an indicator of pulmonary congestion.

Metabolic measurements and assessment of body composition

See data supplement for further details. Briefly, body composition, including fat mass and lean tissue mass, was measured by non-invasive quantitative magnetic resonance imaging (EchoMRI-700, Echo Medical System, Houston, TX). During the last week of the experiment, mice were subjected to a cold tolerance test (CTT). Glucose tolerance tests (GTT) and non-fasting blood glucose levels were measured using tail vein blood samples using an Accu-Chek glucometer (Roche Diagnostics Corp, Indianapolis, IN). For details of CTT and GTT, see methods supplement.

Cell preparation and flow cytometry

Stromal vascular fraction (SVF) from epididymal WAT was isolated according to Orr's protocol²⁵. Cell staining was performed as previously described²⁶. See methods supplement for details.

Gene expression analysis by qRT-PCR

See online methods and Supplemental Table 1 for details.

Western blot analysis

Protein extraction and western blot were performed as previously described¹⁶. See data supplement for details.

Statistical Analysis

Data are shown as mean±SEM unless otherwise stated. Statistical significance of differences between two groups was assessed using the Student's *t*-tests (2-sided). In those cases when data were not sampled as a normal distribution, non-parametric Mann-Whitney test was used. Results of GTT and CTT experiments were evaluated by two-way repeated measures ANOVA. *P* 0.05 values were considered significant. All statistical tests were performed using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA).

Results

Murine model of HFpEF

Characteristics and echocardiography parameters are presented in Tables 1 and 2. Weekly BP and HR are shown in Supplemental Figure 1A. HFpEF mice demonstrated significantly increased systolic and diastolic BP (SBP and DBP) by the end of 4 weeks of aldosterone infusion. HR was comparable between Sham and HFpEF (Table 1). LV structure, systolic and diastolic functions are shown in Table 2. LVEF was preserved and there was evidence of lung congestion in HFpEF mice (Tables 1 and 2). HFpEF mice demonstrated increased LV mass and total wall thickness (TWT) compared to Sham (*P*<0.001). Similarly, the heart weight to body weight ratio was also increased in HFpEF (*P*<0.001 *vs.* Sham). Although there was a trend to smaller LV cavity size in HFpEF mice it was not significantly different to Sham. Relative wall thickness (RWT) was increased in HFpEF mice (*P*<0.01 *vs.* Sham). At similar HR, HFpEF mice showed severe diastolic dysfunction (DD) (Table 2). Both mitral E and A velocities were elevated in HFpEF mice (*P*<0.01 *vs.* Sham; Supplemental Figure 2). The increased A velocities indicates that LV filling was increasingly dependent on an increase in atrial pressure to drive blood into the LV, during diastole. As early diastolic transmitral pressure gradient increased, early diastolic flow velocities also rose, and the E wave increased, causing the E/A ratio to “normalize”. Similar to humans, where isovolumic relaxation time (IVRT) initially increases with impaired relaxation but then decreases with progressive worsening of diastolic function, IVRT was significantly shorter (*P*<0.05 *vs.* Sham) and deceleration time (DT) was significantly decreased in HFpEF mice (*P*<0.001 *vs.* Sham). In addition to DD, there was evidence of HF with the preservation of LVEF after 4 weeks of *d*-aldosterone infusion.

Body composition, characteristics and glucose levels

Body weights were comparable between both groups during the 4 weeks of HFpEF induction (Table 1 and Supplemental Figure 1B). Magnetic resonance imaging (MRI) analysis, performed to assess total lean and fat mass, showed no difference between HFpEF

and Sham mice (Figure 1A). Measurements of WAT weights however, showed that both epididymal (eWAT) and inguinal (iWAT) adipose depots were significantly smaller in HFpEF mice *vs.* Sham (eWAT: 7.59 *vs.* 10.67 mg/g and iWAT: 6.34 *vs.* 8.38 mg/g; Table 1 and Figure 1B, *upper and middle panel*). Importantly, there was a relative increase in brown adipose tissue (BAT) weight in HFpEF mice *vs.* Sham (5.96 *vs.* 4.50 mg/g; $P<0.05$) (Table 1 and Figure 1B, *lower panel*).

Although there was no evidence of ascites in HFpEF mice, spleen weight was significantly increased in HFpEF *vs.* Sham ($P<0.001$). Similarly, there was a trend towards increased liver mass ($P=NS$ *vs.* Sham). Skeletal muscle weight measurement, specifically gastrocnemius muscle, was smaller in HFpEF mice ($P<0.05$ *vs.* Sham). Glucose levels were comparable in both experimental groups under similar non-fasting and fasting conditions (Table 1 and Supplemental Figure 1C). Similarly, glucose metabolism, as determined by the GTT, was similar in HFpEF and Sham mice (Supplemental Figure 1C).

Adipose tissue morphology

To examine whether weight changes in adipose depots were due to morphological alterations, eWAT, iWAT and BAT histological analysis were performed (Figure 1B). HFpEF mice demonstrated significant alterations in WAT morphology. There was a shift to smaller adipocyte size in both eWAT (867.91 *vs.* 986.71 μm^2 ; $P<0.01$) and iWAT in HFpEF mice (858.43 *vs.* 1006.22 μm^2 ; $P<0.05$) compared to Sham. The smaller adipocytes had multilocular lipid droplets, a typical feature of WAT undergoing beiging or brown-like AT (Figure 1C).

Phenotype switch of adipose tissue in HFpEF

We sought to investigate molecular changes that may accompany the morphological changes observed in WAT. There was a marked phenotype switch in eWAT and iWAT in HFpEF mice, with a significant increase in beiging transcripts such as *ucp-1*, *cidea* and *eva* by 5-, 2- and 3-fold, respectively in eWAT and 9-, 2- and 3-fold in iWAT (Figures 2A and 2B). Conversely, there was a significant decrease in mRNA levels of typical white adipose markers such as *leptin*, *resistin*, and even *adiponectin* in the case of eWAT in HFpEF mice (Figure 2A and B).

We next evaluated the molecular phenotype of BAT. Despite the significant increase in BAT weight in HFpEF (Table 1), there was a marked decrease in expression levels of *ucp-1*, *cidea* and *eva* ($P<0.05$ *vs.* Sham; Figure 2C). Furthermore, transcript levels of lipolytic proteins hormone sensitive lipase (*hsl*), lipoprotein lipase (*lpl*) and fatty acid binding protein 4 (*fabp4*) were all significantly decreased in BAT of HFpEF mice ($P<0.05$ *vs.* Sham; Figure 2C). Importantly, these changes were also associated with an impaired thermogenic response in HFpEF mice during the CTT (Figure 2D). Body temperature was initially the same between groups (37.3°C) but, core body temperature dramatically decreased in HFpEF mice after 4 hours of cold (27.8 $\pm$ 1.8 *vs.* 34.1 $\pm$ 0.8 °C; $P<0.05$ *vs.* Sham). Similarly, after only 4 hours of CTT, HFpEF mice had a significant decrease in body weight (−2.1 $\pm$ 0.2 *vs.* −1.2 $\pm$ 0.2 g; $P<0.05$ *vs.* Sham). These data suggest that BAT function may be decreased or impaired, since normal BAT function is expected to improve thermoregulation.

Inflammatory, Fibrotic and Oxidative status in WAT of HFpEF

We further characterize WAT in HFpEF mice by assessing the inflammatory, fibrotic, and oxidative status in WAT. Recent studies showed changes in the inflammatory response in WAT during being and adaptive thermogenesis^{27, 28}. FACS analysis of SVF from eWAT revealed significantly increased neutrophil content in HFpEF mice vs. Sham ($6.25 \times 10^2 \pm 1.2 \times 10^2$ vs. $1.43 \times 10^2 \pm 0.36 \times 10^2$; $P < 0.05$; Figure 3A and B). Ly6C^{high} monocytes (pro-inflammatory function), Ly6C^{low} macrophages (patrolling behavior), and Ki67⁺ Ly6C^{low} macrophages (macrophage proliferation), were comparable between HFpEF and Sham mice. We also analyzed the inflammatory state in iWAT by qRT-PCR. Similar to the results obtained in eWAT, there was greater ly6g gene expression (indicating an increase in neutrophil infiltration) in this adipose depot in HFpEF mice ($P < 0.05$ vs. Sham; Figure 3C). There were no differences in f4/80, tnfa and mcpl transcript expression in iWAT between HFpEF and Sham mice (Figure 3C). There were also no differences in fibrotic (collagen 1, 3 and 6) and oxidative markers (NAD(P)H subunits p22^{phox} and p47^{phox}) in eWAT or iWAT in HFpEF vs. Sham mice (Supplemental Figure 3). Similar results were found in BAT (Figure 3D and Supplemental Figure 3).

Mechanisms implicated in AT phenotype changes

In order to further investigate being activation in WAT during HFpEF, we measured p38 MAPK activation in eWAT since it was previously shown that phosphorylation of p38 MAPK can induce UCP-1 protein expression^{23, 29}. In eWAT, p38 MAPK phosphorylation was increased by $137 \pm 27\%$ in HFpEF compared to Sham mice ($P < 0.01$; Figure 4A). Furthermore, this increase in p38 MAPK expression was accompanied by an increase in HSL phosphorylation in eWAT of HFpEF mice ($P < 0.05$ vs. Sham; Figure 4B). With the increase in p38 MAPK activation seen in eWAT, we sought to address the upstream pathway leading to p38 MAPK activation. We first evaluated $\beta 3$ -adrenergic expression, which has been shown to be related to being activation^{27, 30}. In eWAT of HFpEF mice $\beta 3$ -receptor protein expression was diminished by $-35 \pm 7\%$ compared to Sham ($P < 0.05$; Figure 4C). However, PKA phosphorylation, an important downstream target of the $\beta 3$ -adrenergic pathway, was not altered in eWAT of either HFpEF or Sham mice (Figure 4D). We sought to determine natriuretic peptide receptors expression in eWAT and BAT. In eWAT, the ratio of gene expression of the natriuretic peptide receptor type A (npa: active form) to natriuretic peptide receptor type C (nrc: clearance receptor) was increased in HFpEF compared to Sham mice ($P = 0.001$; Figure 4E), but not different between these groups in BAT (Figure 4F). These results were also accompanied by an increase in myocardial, left ventricular anp and bnp expression by 2.7- and ~2-fold, respectively in HFpEF mice, as previously described¹⁶ ($P < 0.001$ and $P < 0.05$, respectively vs. Sham; Figure 4G).

In order to ensure that these findings were not a model-specific finding, i.e. an effect of chronic aldosterone, a group of WT mice were subjected to transverse aortic constriction (TAC) or sham procedure. Two weeks after TAC, mice developed HFpEF and showed echocardiographic features consistent with DD (Supplemental Table 2). TAC mice demonstrated higher E velocity and higher E/A ratio consistent with impaired DD. TAC mice had increased LV mass, TWT and RWT ($P < 0.05$ vs. Sham). LVEF was preserved. TAC mice had significantly increased lung congestion (data not shown).

Body weight was comparable in TAC and Sham-operated mice (28.16 ± 0.47 vs. 27.61 ± 1.09 g; Figure 5A). Evaluation of WAT from TAC was similar to results observed in aldosterone-infused HFpEF mice (Figure 5B). WAT depot had smaller adipocytes (Figure 5C–D). Moreover, WAT from TAC mice showed elevated transcript levels of *ucp-1* ($P < 0.05$) and trends to increased *cidea* and *eva* expression ($P = 0.07$ and $P = 0.07$, respectively). Conversely leptin and resistin expression was downregulated ($P < 0.05$ and $P = 0.06$, respectively) (Figure 5E). Similarly, the ratio of *npra/nprc* in WAT was increased in TAC mice ($P < 0.05$ vs. Sham; Figure 5F). This was due to increases in *npra* expression in TAC (data not shown).

Discussion

In this study, we show that HFpEF is associated with changes in AT in non-overweight mice. Aldosterone-infused mice developed HTN, LV hypertrophy, DD and lung congestion, while maintaining a preserved LVEF, thus resulting in HFpEF^{15, 16, 24}. Similar changes were seen in TAC-induced HFpEF. There were no changes in total body weight or modifications in total lean and fat mass. However, there was a shift in WAT and BAT weight, with a marked decrease in white adipose depots while the brown depot was significantly increased. The macroscopic decrease of WAT mass was associated with a reduction in adipocyte size in both visceral (eWAT) and subcutaneous (iWAT) AT. The smaller adipocyte size in WAT was associated with the presence of brown-like structures, islets composed of small adipocytes with large nuclei and multilocular cytoplasm. Clusters of UCP-expressing adipocytes have been shown to develop in WAT in response to various stimuli such as sympathetic activation³⁰. These adipocytes have been named “beige” or “brite” (brown-in-white). Similar to adipocytes in BAT, beige cells are defined by their multilocular lipid droplet morphology, high mitochondrial content and the expression of a core set of brown fat-specific genes (such as *ucp1*, *cidea* or *eva*)³¹. We showed an increase in these brown specific markers, in both eWAT and iWAT in HFpEF mice. Furthermore, there was a reduction in classical murine white adipose markers such as leptin, adiponectin and resistin³². Together, these indicate that HFpEF is associated with the activation of beiging in WAT. Similar findings were seen in TAC-induced HFpEF mice.

When HFpEF mice were challenged in a cold tolerance experiment, they showed an ineffective response of shivering thermogenesis. This failure could indicate an inability to maintain shivering, but may also indicate an inadequate function of BAT³⁰. BAT, in contrast to *bona fide* WAT, dissipates energy through uncoupled respiration and heat production (thermogenesis). This is mediated by the major thermogenic factor UCP-1 and can be activated by stimuli, such as cold exposure and adrenergic factors^{33–35}. Although WAT depots possess the capacity to acquire brown fat characteristics in response to thermogenic stimuli, it is more likely that the beiging of WAT observed here is due to increased natriuretic peptides, since circulating levels of BNP and ANP are elevated in HF². Analysis of BAT molecular markers also showed a decrease in browning markers that were also accompanied by decreased expression of lipolytic enzymes, such as *hsl*, *lpl* and *fatp4*, which are responsible for AT regulation of lipid breakdown and the generation of free fatty acids³². Thus, the effects of the natriuretic peptides on WAT were also associated with alterations in BAT machinery. It appears that functional and molecular changes occurring in active BAT depots are also present in adult humans and can be readily activated when exposed to

stimuli, like mild cold^{34–36}. Beiging of WAT is usually associated with beneficial effects such as an improvement of insulin sensitivity, or amelioration of obesity^{31, 37, 38}. However, recent studies suggest that this process may not always be beneficial. Amongst a number of such settings, beiging of WAT is thought to be a driver for weight loss during cancer-associated cachexia²⁷. In the same way, our data suggest that the activation of beiging in WAT appears to be inadequate in HFpEF. Similarly, although we have not proven cause, there is a parallel downregulation of the BAT transcripts that are important in fuel generation and thermal homeostasis.

Interestingly, the changes observed in WAT were not accompanied by significant changes in fibrosis or oxidative stress status as we might expect in response to HFpEF^{39, 40}. Notably, neutrophil content was higher in both eWAT and iWAT. Neutrophils, are short-lived cells, and are recognized as primary effector cells in acute inflammatory responses and participate in the initiation of immune responses and resolution of inflammation⁴¹. In both obese humans and animal models, neutrophil-derived proteins, such as neutrophil elastase or myeloperoxidase, and markers for neutrophil activation and neutrophil infiltration are increased^{42–44}. Thus, although neutrophils are implicated in the modulation of AT inflammation in the early stages of obesity, their presence in AT in response to a high fat diet may last up to 90 days⁴³. Our results therefore suggest that the onset of immune activation in WAT may be related to ongoing changes, such as continued neutrophil infiltration in WAT, setting the stage for tissue infiltration by other immune cells, such as macrophages.

Several mechanisms are implicated in WAT beiging, including prolonged cold exposure and adrenergic activation, that both converge with p38 MAPK activation^{27, 29}, as well as increases in VEGF^{45, 46}. In HFpEF mice, p38 MAPK phosphorylation was increased in WAT and this increase was associated with an elevation in HSL phosphorylation, indicating increased AT lipolysis²⁸. Adrenergic signaling is classically thought to be the principal activator of thermogenic machinery. Elevated catecholamines increase intracellular cAMP levels via the β 3-receptor, directly activating cAMP-dependent protein kinase (PKA), which can also phosphorylate kinases in the p38 MAPK pathway⁴⁷. However, in HFpEF mice there was downregulation of β 3-receptor expression and PKA phosphorylation indicating that the β 3 pathway was not implicated in the changes observed in our experimental model. It is well known that BAT is densely innervated, and consequently, thermogenesis in this tissue is regulated primarily by adrenergic tone. In contrast, white adipose depots are less extensively innervated, suggesting that alternate pathways may control p38 MAPK activation and subsequent UCP-1 expression⁴⁸. We thus focused on other effectors that could be related to the observed changes. Bordecchia *et al.*, showed that cardiac natriuretic peptides mediates beiging in WAT via the p38 MAPK pathway²³. Similarly, we examined natriuretic peptide receptors in adipose depots and found that the ratio of np_{ra} to np_{rc}, was increased in eWAT of HFpEF mice indicating increased signaling and/or decreased clearance, and demonstrating that natriuretic peptides may play a role in p38 MAPK activation. Furthermore, anp and bnp expression levels, which are primarily synthesized in the heart with increased expression in HFpEF^{16, 40}, were increased in the LV from mice with HFpEF. We thus propose that the natriuretic peptides secreted from the heart play an

important role in the changes observed in WAT and this pathway may be a target for drug development in HFpEF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Clinical Perspective

The heart and adipose tissue (AT) are linked via the well-documented association of obesity and heart diseases such as coronary artery disease and hypertension. Obesity is a comorbidity that is also highly associated with heart failure with preserved ejection fraction (HFpEF). Increasing evidence demonstrates that the heart can directly regulate AT via cardiokines, but their role in mediating crosstalk between the heart and AT is incompletely understood. This study demonstrates that during HFpEF, there was an attempt of white AT (WAT) to turn on a “beiging” program. This novel role of WAT during HFpEF might provide further insight into how AT is regulated in HFpEF. Thus, an attractive model is that AT, as an endocrine organ, also responds and regulates the pathological cardiac phenotype. Others have described the effect of natriuretic peptides on WAT in relation to metabolism, but to our knowledge not in HFpEF with changes seen also in brown AT. These data have implications for HFpEF and future drug therapies, where there continues to be a significant need, given the negative human HFpEF clinical trials to date. This study emphasizes the importance of crosstalk between the heart and AT and raises new insights into the pathogenesis of HFpEF.

Figure 1. HFpEF induces changes in adipose tissue depots size and morphology

A) Body composition assess by MRI, including fat and lean mass, in Sham and HFpEF mice 4 weeks after saline or *d*-aldosterone infusion (n=6–12 animals/group). **B)** Representative macroscopic and microscopic (H&E staining, 20 $\times$) images of eWAT (*Upper Panel*) and iWAT (*Middle Panel*). Representative macroscopic and microscopic (H&E staining, 40 $\times$) images of BAT (*Lower Panel*) from Sham and HFpEF mice (n=4–8 animals/group). **C)** Adipocyte size of eWAT and iWAT.

Figure 2. qRT-PCR analysis of adipose depots and cold tolerance test in Sham and HFpEF mice
 Gene expression analysis of brown (*ucp-1*, *cidea* and *eva*) and white (*adiponectin-apn*, *leptin* and *resistin*) adipose tissue markers in eWAT (**A**) and iWAT (**B**) (n=6–12 animals/group).
C) Gene expression analysis of brown adipose tissue markers (*ucp-1*, *cidea* and *eva*) and metabolic enzymes (hormone sensitive lipase: *hsl*, lipoprotein lipase: *lpl* and fatty acid binding protein 4: *fatp4*) in BAT (n=6–12 animals/group). **D**) *Left Panel*: Basal body temperature prior to the cold tolerance test (CTT); *Middle Panel*: body temperature during CTT; *Right Panel*: loss of body weight after 4 hours of CTT (n=6–12 animals/group).

A.

B.

C.

D.

Figure 3. Inflammatory markers in adipose depots of Sham and HFpEF mice

A) Number of neutrophils, Ly6C^{low} macrophages, and Ly6C^{high} monocytes per mg of eWAT and Ki67+ Ly6C^{low} macrophages proliferation. **B)** Representative flow cytometry staining of SVF cells from eWAT. **C)** qRT-PCR analysis of inflammatory markers in iWAT. **D)** qRT-PCR analysis of inflammatory markers in BAT. (n=6–12 animals/group).

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Figure 4. Mechanisms involved in white adipose tissue phenotypic change during HFpEF

Quantitative analysis and representative blots of **A)** p38 total protein and p38 phosphorylation, **B)** HSL total protein expression and HSL phosphorylation, **C)** β 3-receptor protein and **D)** PKA total protein and PKA phosphorylation in eWAT from Sham and HFpEF mice. **E)** npna/npnc gene expression ratio in eWAT and **F)** BAT from Sham and HFpEF mice. **G)** anp and bnp left ventricular gene expression in Sham and HFpEF mice. (n=4–14 animals/group).

Figure 5. HFpEF induced by TAC recapitulates the findings observed in white adipose tissue depots size and morphology

A) Body weight and **B)** white adipose tissue weight (WAT) two weeks after transverse aortic constriction (TAC) or Sham operation. **C)** Adipocyte size and **D)** representative microscopic (H&E staining, 20x) images of WAT in TAC and Sham mice. **E)** Gene expression analysis of brown (*ucp-1*, *cidea* and *eva*) and white (adiponectin-*apn*, leptin and resistin) adipose tissue markers, and **F)** *npra/nprc* gene expression ratio in WAT from Sham and TAC-HFpEF mice. (n=4 animals/group). * $P=0.05$ vs. Sham; ** $P<0.05$ vs. Sham.

Table 1

Characteristics of Sham and HFpEF mice after 4 weeks of Saline or *d*-Aldosterone infusion.

	Sham n=6	HFpEF n=12
Systolic blood pressure (mm Hg)	129±3	146±3 ^{**}
Diastolic blood pressure (mm Hg)	89±6	114±4 ^{**}
Heart rate (beats/min)	666±29	623±16
Body weight (g)	26.6±1.0	26.3±0.4
Heart weight/Body Weight (mg/g)	3.45±0.07	4.01±0.09 ^{***}
Wet lung/Dry lung weight ratio	4.01±0.10	4.77±0.10 ^{**}
Spleen weight/Body weight (mg/g)	3.29±0.09	3.77±0.08 ^{***}
eWAT weight/Body weight (mg/g)	10.67±0.95	7.59±0.57 ^{**}
iWAT weight/Body weight (mg/g)	8.38±0.84	6.34±0.47 [*]
BAT weight/Body weight (mg/g)	4.50±0.34	5.96±0.42 [*]
Gastrocnemius weight/Body weight (mg/g)	5.66±0.16	4.90±0.22 [*]
Liver weight/Body weight (mg/g)	44.81±1.68	47.45±1.40
Non-fasting glucose levels (mg/dL)	203.0±11.2	211.0±13.7

Data are expressed as mean ± SEM. Abbreviations: BAT, brown adipose tissue; eWAT, epididymal white adipose tissue; HFpEF, heart failure preserved ejection fraction; iWAT, inguinal white adipose tissue. (n=6–12 mice per group)

* P<0.05 vs. Sham;

** P<0.01 vs. Sham;

*** P<0.001 vs. Sham.

Table 2

Echocardiographic Parameters of Sham and HFpEF mice after 4 weeks of Saline or *d*-Aldosterone infusion.

	Sham n=6	HFpEF n=12
<i>LV Structure and Systolic Function</i>		
LV mass (mg)	89.43±3.35	134.8±6.26***
Total wall thickness (mm)	0.83±0.03	1.11±0.05***
LV end- systolic diameter (mm)	1.66±0.16	1.36±0.12
LV end- diastolic diameter (mm)	3.34±0.13	3.19±0.13
LV ejection fraction (%)	82.18±3.05	87.81±2.05
Relative wall thickness (RWT)	0.50±0.04	0.72±0.05**
<i>Diastolic Function</i>		
Mitral E velocity, mm/s	664±45	1304±150**
Mitral A velocity, mm/s	498±70	1190±135**
E/A	1.28±0.09	1.11±0.02*
Deceleration time (ms)	22.67±1.65	14.57±0.90***
Isovolumic relaxation time (ms)	20.36±1.10	17.6±1.21*

Data are expressed as mean±SEM. Abbreviations: IVS, intra-ventricular septum; LV, left ventricle. (n=6–12 mice per group)

* P<0.05 vs. Sham;

** P<0.01 vs. Sham;

*** P<0.001 vs. Sham.