Sexual dimorphism in obesity-mediated left ventricular hypertrophy

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Submitted 6 August 2012; accepted in final form 8 May 2013

Böhm C, Benz V, Clemenz M, Sprang C, Höft B, Kintscher U, Foryst-Ludwig A. Sexual dimorphism in obesity-mediated left ventricular hypertrophy. Am J Physiol Heart Circ Physiol 305: H211–H218, 2013. First published May 10, 2013; doi:10.1152/ajpheart.00593.2012.—In the present study we investigated the influence of sex difference on the development of left ventricular hypertrophy (LVH) during obesity. Male and female C57BL/6J mice were fed for 15 and 25 wk with a high-fat diet (HFD) or low-fat control diet (LFD). Analysis of body composition, monitoring of body weight (BW), and echocardiographic analysis were performed, as well as analysis of expression of different adipocytokines in epicardial adipose tissue. The increment in left ventricular mass (LVM) after HFD (25 wk) was significantly stronger in male mice compared with female mice [LVM: male, 116.9 ± 2.9 (LFD) vs. 142.2 ± 9.3 mg (HFD); female, 84.3 ± 3.3 (LFD) vs. 93.9 ± 1.7 mg (HFD)]. Bsex < 0.01. In parallel, males developed a higher BW and fat mass after 25 wk HFD than female mice [BW: male, 33 ± 0.9 (LFD) vs. 53 ± 0.8 g (HFD); fat mass: male, 8.8 ± 0.9 (LFD) vs. 22.8 ± 0.7 g (HFD); BW: female, 22.5 ± 0.4 (LFD) vs. 33.7 ± 1.3 g (HFD); fat mass: female, 4.0 ± 0.2 (LFD) vs. 13.2 ± 1.2 g (HFD)] (P < 0.01 for BW and fat mass). The mRNA expression of adipocytokines in epicardial fat after 25 wk of diet showed higher levels of adiponectin (2.8-fold), leptin (4.2-fold), and vaspin (11.9-fold) in male mice compared with female mice (P < 0.05). To identify new adipose-derived mediators of LVH, we further elucidated the cardiac impact of vaspin. Murine primary cardiac fibroblast proliferation was significantly induced by vaspin (1.8-fold, vaspin 1 nM). Murine primary cardiac fibroblast proliferation was significantly induced by vaspin (1.8-fold, vaspin 1 nM) compared with 1.9-fold induction by angiotensin II (10 nM). Analysis of body composition, monitoring of body weight (BW), and echocardiographic analysis were performed, as well as analysis of expression of different adipocytokines in epicardial adipose tissue. The increment in left ventricular mass (LVM) after HFD (25 wk) was significantly stronger in male mice compared with female mice [LVM: male, 116.9 ± 2.9 (LFD) vs. 142.2 ± 9.3 mg (HFD); female, 84.3 ± 3.3 (LFD) vs. 93.9 ± 1.7 mg (HFD)], Psex < 0.01. In parallel, males developed a higher BW and fat mass after 25 wk HFD than female mice [BW: male, 33 ± 0.9 (LFD) vs. 53 ± 0.8 g (HFD); fat mass: male, 8.8 ± 0.9 (LFD) vs. 22.8 ± 0.7 g (HFD); BW: female, 22.5 ± 0.4 (LFD) vs. 33.7 ± 1.3 g (HFD); fat mass: female, 4.0 ± 0.2 (LFD) vs. 13.2 ± 1.2 g (HFD)] (P < 0.01 for BW and fat mass). The mRNA expression of adipocytokines in epicardial fat after 25 wk of diet showed higher levels of adiponectin (2.8-fold), leptin (4.2-fold), and vaspin (11.9-fold) in male mice compared with female mice (P < 0.05). To identify new adipose-derived mediators of LVH, we further elucidated the cardiac impact of vaspin. Murine primary cardiac fibroblast proliferation was significantly induced by vaspin (1.8-fold, vaspin 1 nM), P < 0.05 vs. control) compared with 1.9-fold induction by angiotensin II (10 μM). The present study demonstrates a sex-dependent regulation of diet-induced LVH associated with sexual dimorphic expression of adipocytokines in epicardial adipose tissue.

LEFT VENTRICULAR (LV) hypertrophy (LVH) has been established as an independent cardiovascular risk factor. The etiology of LVH is multifactorial and often a consequence of chronically elevated blood pressure. In addition to arterial hypertension, other factors have been described to initiate and maintain LVH such as vascular diseases, genetic diseases, etc. (10). Obesity is worldwide, increasing together with obesity-associated diseases such as insulin resistance, diabetes mellitus type 2, and dyslipidemia (35, 36). In the last years, obesity has led to the identification of new molecular and therapeutic targets for both sexes. It is now known that the development of overweight and obesity, as well as the (patho-) biology and distribution of adipose tissue underlies a sex-dependent regulation, subsequently causing sex differences in obesity-mediated metabolic disease (4, 5, 9, 16). However, the relevance of these metabolic differences for sexual dimorphisms of cardiac disease, in particular of cardiac hypertrophy, is not well defined.

The present study investigated the impact of sex on the development of obesity-mediated cardiac hypertrophy and aimed to identify new molecular mediators of obesity-mediated LVH based on sexual dimorphisms. Therefore, female and male C57Bl/6J mice were fed with a high-fat diet (HFD) or low-fat control diet (LFD) for 15 and 25 wk, and the development of cardiac hypertrophy was analyzed by repetitive echocardiography during the course of overweight/obesity. In addition, molecular analysis was performed in epicardial adipose tissue.

METHODS

Animals. Male and female C57Bl/6J mice (4–6 wk old) were housed under constant environmental conditions, 20–24°C temperature, 55 ± 10% relative humidity, and 12-h:12-h light-dark cycle. Mice were fed ad libitum with either HFD (60% kcal from fat, D12492, Research Diets) or LFD (10% kcal from fat, D12450B). Body weight (BW) was monitored throughout the experiment. Mice were randomly assigned to the following groups: male HFD, female HFD, male LFD, and female LFD. After 10, 15, 20 and 25 wk of diet, all animals underwent an echocardiographic analysis, and after 15 and 25 wk of diet, the specific groups underwent a body composition analysis [nuclear magnetic resonance (NMR)] and were euthanized under isoflurane anesthesia by cervical dislocation. The organs were dissected and shock frosted in liquid nitrogen and stored at −80°C. All animal procedures were performed according to the guidelines of the Charité-Universitätsmedizin Berlin and were approved by the Landesamt für Gesundheit und Soziales (Berlin, Germany) for the use of Pharmacology and Metabolic Research, Hessische Str. 3-4, 10115 Berlin, Germany (e-mail: anna.foryst@charite.de).

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of laboratory animals and according to the current version of the German Law on protection of animals.

**Echocardiography.** Echocardiographic analysis was performed after 10, 15, 20, and 25 wk, using a Vevo 770 high-resolution imaging system (VisualSonics, Toronto, ON, Canada) with a RMV 707 scan head. The mice were initially anesthetized with 3% isoflurane (inhalation); fixed on a heated pad at 37°C, stabilizing their temperature; and continuously monitored by ECG recording. During the analysis isoflurane was reduced to 2–1.5%. Ultrasonic transmission gel was used to scan the parasternal short axis of the heart in B- and M-mode. The thickness of the posterior and anterior wall, as well as the intraventricular diameter, was determined in both systole and diastole. Diastolic and systolic parameters are shown. Additional parameters were calculated from these data according to the manufacturer’s instruction (Vevo 770, Standard Measurements and Calculations).

**NMR analysis.** Body composition was determined in conscious mice after 15 and 25 wk using a NMR whole body composition analyzer (Echo Medical Systems, Houston TX). We performed two measurements without anesthesia according to the manufacturer’s instruction.

**Tail-cuff blood pressure.** Systolic blood pressure was measured after 15 wk of HFD using a computerized, noninvasive, tail-cuff system (Power Lab 4/20 with tail-cuff MLT125/M, both from ADinstruments).

**RNA analysis.** Total RNA from epicardial fat was isolated using Qiazol and the RNAs easy Micro Kit from Qiagen according to the manufacturer’s instructions. The RNA was DNAs e free DNase set (Qiagen) and reverse transcribed using reverse transcriptase, RNAsin, and dNTPs (Promega) according to the manufacturer’s instructions.

**Quantitative RT-PCR.** Quantitative real-time PCR was performed using an MxPro-system from Stratagene. Quantitative RT-PCR was performed in the presence of an intercalating fluorescent dye (SYBR Green, Life Sciences) when performed with primers and in presence of a labeled probe when performed with an assay on demand (Life Science). Reference dye glycine conjugate of 5-carboxy-X-rhodamine was used as a passive control, and the relative amount of RNA was calculated by normalization to 18s ribosomal RNA. The primer sequences used are available on demand.

**Cardiac fibroblast isolation.** Hearts of C57Bl/6J mice were isolated, cleaned from blood with PBS, and minced into small pieces. These were sequentially lysed with collagenase-dispase (Roche). Enzyme activity was neutralized using DMEM with 10% FBS and 1% penicillin-streptomycin. The resulting cell suspension was allowed to settle for 1 h in 75-cm² cell culture flasks, resulting in cardiac fibroblasts attached to the surface of the flask. Immunohistochemical evidence for purity of cardiac fibroblasts culture by using this method has been recently provided (13, 19).

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**Fig. 1.** A: body weight (BW) responses of female (f) and male (m) mice to high-fat diet (HFD) and low-fat diet (LFD). The body weight was determined twice per week and is pictured as means ± SE. Factor interaction for BW: *P_{sex×diet} < 0.001; 2-way ANOVA/repeated measurements (n = 31–34 mice/group 0–15 wk, n = 14–15 mice/group 15–25 wk). B: fat mass in percentage of total BW after 15 and 25 wk. NMR data from different groups adjusted to BW (n = 8–10 mice group); factor interaction for fat mass, *P_{diet} < 0.001; #P_{sex×diet} < 0.05 (2-way ANOVA with Bonferroni post-test).
Cardiac fibroblast proliferation assay. Cardiac fibroblast proliferation was determined using a bromodeoxyuridine (BrdU) chemiluminescent cell proliferation ELISA (Roche). Cells were plated in black 96-well plates 5,000 cells each. They were cultured for 24 h in full media, starved overnight, and stimulated for 24 h. Afterward, a labeling period of 24-h measurements was performed according to the manufacturer’s protocol with a Bio-Rad Benchmark Plus microplate reader.

Furthermore, free fatty acids were measured with nonesterified fatty acid kit (WAKO), and insulin and leptin levels were determined using an ELISA (Millipore). Blood glucose was measured with a fatty acid kit (WAKO), and insulin and leptin levels were determined according to the manufacturer’s protocol with a Bio-Rad Benchmark Plus microplate reader.

RESULTS

BW and fat mass. Under LFD and HFD, BW continuously increased in both sexes (Fig. 1A). HFD-fed animals exhibited a significantly stronger BW gain than LFD-fed mice. BW response to HFD was significantly augmented in male mice compared with female mice (factor interaction, \( P_{\text{sex\times time}} < 0.001 \)) (Fig. 1A). In parallel, an increase of fat mass under HFD was detected in both sexes (Fig. 1B). Whereas male mice showed a fast response to HFD with major fat mass gain during the first 15 wk (5.8 \( \pm \) 0.4 g after feeding with LFD and 20.2 \( \pm \) 0.5 g under HFD), females had a delayed HFD response and increased their fat mass mainly between week 15 and 25 (from 4.0 \( \pm \) 0.2 g feeding with LFD to 13.2 \( \pm \) 1.2 g feeding with HFD) (Fig. 1B). Also, lean mass increased during the course of the experiment in both sexes. However, in contrast to fat mass, lean mass only increased 12% in females and 26% in males after 25 wk of HFD compared with LFD (Table 1).

Echocardiography. To determine whether there is an influence of sex and diet on the progression of cardiac hypertrophy, echocardiographic analysis was performed during the feeding protocol as outlined in METHODS. Male mice developed a marked cardiac hypertrophic response to HFD feeding with a significant difference between LFD: LV mass (LVM) at 25 wk, 116.9 \( \pm \) 2.9 mg; and HFD: LVM at 25 wk, 142.2 \( \pm \) 9.3 mg \(( P < 0.01 \)) (Fig. 2 and Table 2). In contrast, the female cardiac response to HFD feeding was attenuated compared with males showing a mild LVM increase in the HFD group after 25 wk [93.9 \( \pm \) 1.7 mg; \( P = \) not significant (NS)] compared with the LFD group (843 \( \pm \) 3.3 mg) (Fig. 2 and Table 2). LVM data corrected for tibia length (TL) showed similar results (Table 2). The interaction between sex and diet during HFD feeding (0–25 wk) was highly significant indicating, a sex-dependent regulation of HFD-induced cardiac hypertrophy. No significant differences between diet and sex were documented for heart rate, LV ejection fraction (in %), systolic values of LV internal dimension, percent LV fractional shortening, as well as wall thickness (Table 2). As BW and lean mass varied in a diet-dependent manner (Table 1), the results obtained from the normalization of LVM (or heart weight) to BW (or lean mass) are difficult to interpret. Therefore, the normalization to TL seems to be more reliable.

As cardiac hypertrophy often results from increased blood pressure, the mean systolic arterial blood pressure was measured in female and male animals after 15 wk of HFD (Fig. 3B). After 15 wk of HFD, male mice showed significantly increased LVM, but we observed no differences of blood pressure measured in both sexes. Similar to results presented in Fig. 2, gravimetric LVM, measured in those animals, was significantly increased in males, compared with female littermate (Fig. 3, C and D).

Adipocytokine expression in epicardial fat. Epicardial adipose tissue has been recently characterized as a crucial paracrine regulator of LV remodeling under pathological conditions (1, 3, 7, 27). In the present model, only mice under HFD developed epicardial adipose tissue. To investigate the mediators involved in the development of HFD-dependent cardiac hypertrophy, we first analyzed the standard blood and serum parameters of the female and male mice fed with HFD (Table 3). When compared with LFD-littermates, both female and male

<table>
<thead>
<tr>
<th>Table 1. Body composition after 15 and 25 wk of diet (NMR)</th>
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<tr>
<td>Females LFD</td>
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<tr>
<td>----------------------------------------------------------</td>
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<tr>
<td>15 wk of diet</td>
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<tr>
<td>Body wt, g ( ^* )</td>
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<td>Fat mass, g ( ^* )</td>
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<td>Lean mass, g ( ^* )</td>
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<td>25 wk of diet</td>
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<td>Body wt, g ( ^* )</td>
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<td>Fat mass, g ( ^* )</td>
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<td>Lean mass, g ( ^* )</td>
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Values are means (calculated from absolute mass) \( \pm \) SE; \( n = 9–18 \) mice/group. NMR data performed after 15 and 25 wk of diet. LFD, low-fat diet; HFD, high-fat diet. Factor interaction: \( {^*P_{\text{sex\times diet}}} < 0.001 \) and \( {^{0.001}P_{\text{sex\times diet}}} < 0.01 \) (2-way ANOVA).
Table 2. Echocardiographic measurements

<table>
<thead>
<tr>
<th></th>
<th>Females LFD</th>
<th>Females HFD</th>
<th>Males LFD</th>
<th>Males HFD</th>
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<tbody>
<tr>
<td>IVSd, mm*</td>
<td>0.7 ± 0.03</td>
<td>0.7 ± 0.02</td>
<td>0.7 ± 0.01</td>
<td>0.8 ± 0.02</td>
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<tr>
<td>LVIDd, mm*</td>
<td>3.9 ± 0.07</td>
<td>4.0 ± 0.06</td>
<td>4.4 ± 0.11</td>
<td>4.5 ± 0.14</td>
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<tr>
<td>LV PWd, mm*&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.6 ± 0.01</td>
<td>0.7 ± 0.02</td>
<td>0.7 ± 0.02</td>
<td>0.8 ± 0.02</td>
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<tr>
<td>LVID, s</td>
<td>2.9 ± 0.29</td>
<td>2.9 ± 0.32</td>
<td>3.2 ± 0.43</td>
<td>3.2 ± 0.39</td>
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<tr>
<td>LV %FS</td>
<td>25.6 ± 5.40</td>
<td>27.9 ± 6.09</td>
<td>26.8 ± 7.20</td>
<td>28.2 ± 2.79</td>
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<tr>
<td>LV EF, %</td>
<td>50.5 ± 2.9</td>
<td>54.1 ± 2.9</td>
<td>51.8 ± 3.5</td>
<td>54.5 ± 1.5</td>
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<tr>
<td>Wall thickness, mm</td>
<td>1.3 ± 0.11</td>
<td>1.4 ± 0.05</td>
<td>1.4 ± 0.08</td>
<td>1.6 ± 0.11</td>
</tr>
<tr>
<td>Relative wall thickness, %</td>
<td>33.1 ± 3.95</td>
<td>34.4 ± 2.63</td>
<td>33.0 ± 4.04</td>
<td>35.8 ± 3.81</td>
</tr>
<tr>
<td>LV mass (uncorrected), mg&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>84.3 ± 3.3</td>
<td>93.9 ± 1.7</td>
<td>116.9 ± 2.9</td>
<td>142.2 ± 9.3</td>
</tr>
<tr>
<td>LV mass (uncorrected)/TL, mg/mm&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.8 ± 0.19</td>
<td>5.2 ± 0.1</td>
<td>6.5 ± 0.12</td>
<td>8.0 ± 0.52</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>442 ± 7</td>
<td>454 ± 9</td>
<td>450 ± 11</td>
<td>467 ± 10</td>
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Values are means ± SE; n = 19 mice/group. Echocardiographic parameters determined after 25 wk of diet. IVSd, diastolic interventricular septum thickness; LVIDd, left ventricular (LV) diastolic intraventricular diameter; LV PWd, diastolic LV posterior wall thickness; LVIDs, LV systolic intraventricular diameter; LV %FS, LV fraction shortening; LV %EF, LV ejection fraction; LVM, LV mass; LVM/TL, LVM adjusted to tibia length. *P<sub>sex</sub> < 0.005, male vs. female; *P<sub>diet</sub> < 0.005, HFD vs. LFD (2-way ANOVA). Factor interaction for LVM: *P<sub>sex × diet</sub>, 2-way ANOVA with repeated measurements, shown in Fig. 2.

HFD-fed animals showed increased levels of glucose and leptin and reduced levels of free fatty acids. Contrarily, only male mice fed with HFD showed significantly increased insulin serum levels, compared with other mice.

To identify new sex-dependent mechanisms mediating diet-induced cardiac hypertrophy, we investigated the expression level of adipocytokines in epicardial adipose tissue after HFD feeding. As depicted in Fig. 4A, we found different expression patterns in epicardial fat of male and female mice for adiponectin, leptin, and vaspin (visceral adipose tissue-derived serine protease inhibitor), but not for resistin. When compared with that in female mice, adiponectin expression in male epicardial adipose tissue was elevated by 2.7 ± 0.5-fold and leptin by 4.2 ± 0.7-fold after 25 wk of HFD. No significant sex-dependent difference could be detected for resistin expression. The serine protease inhibitor vaspin was expressed 11.9 ± 2.0-fold higher in male epicardial fat compared with female mice (Fig. 4A).

To exclude systemic sexual dimorphisms, vaspin serum levels were assessed. We found no significant difference between HFD-fed female and male mice after 25 wk of diet (Fig. 4B).

**Effects of vaspin on cardiac cells.** To further evaluate the function of sex-dependent vaspin regulation for the development of cardiac hypertrophy, we tested the response of cardiac myoblasts and cardiac fibroblasts to vaspin stimulation. We used tritium-labeled leucine uptake in cardiac H9C2 myoblasts for studying cell hypertrophy. Vaspin had no significant effect on leucine uptake making a prohypertrophic action of vaspin unlikely (Fig. 5A). Vasopressin served as a positive control. In contrast, vaspin stimulation resulted in a significant induction of cardiac fibroblast proliferation (Fig. 5B). When compared with vehicle control, primary mouse cardiac fibro-
blasts incorporated twice as many BrdU when stimulated with vaspin (Fig. 5B). Vaspin’s effect on BrdU incorporation was comparable with 1 nM angiotensin II, an established profibrotic factor.

**DISCUSSION**

The present study demonstrates a sex-dependent regulation of diet-induced LVH associated with sexual dimorphic expression of adipocytokines in epicardial adipose tissue. Male mice exhibited an augmented hypertrophic response to HFD associated with increased expression of vaspin in epicardial adipose tissue compared with female mice. Vaspin significantly induced cardiac fibroblast proliferation.

Sex differences in cardiac hypertrophy have been previously documented in mouse models of physiological and pathological LVH. In response to exercise, female mice develop a greater increase in LVM than male mice (14). We could recently show that the enhanced cardiac response of female mice to exercise is associated with sex-dependent differences in energy substrate mobilization and utilization (8). In addition to physiological hypertrophy, pathological LVH is also regulated in a sexual dimorphic manner. Skavdahl and colleagues (30) demonstrated in a model of transverse aortic constriction that female mice exhibited a significantly reduced heart weight-to-BW ratio compared with males (30). These data are in accordance with the present study, showing a stronger hypertrophic response to the pathological HFD stimuli in male mice and a more protected phenotype in female mice.

We identified a sexual dimorphic expression pattern of adipocytokines in epicardial adipose tissue after 25 wk of HFD. In accordance, sex differences in systemic adipocytokine regulation have been previously described in human and murine plasma (21). Since epicardial adipose tissue may serve as a paracrine organ directly modulating cardiac pathogenesis (18), we focused on this adipose tissue depot to identify

<table>
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<tr>
<th>Table 3. Effects of 15 wk of diet on mouse serum parameters</th>
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<tr>
<td>Females LFD</td>
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<tr>
<td>Glucose, mg/dl</td>
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<tr>
<td>FFA, mM</td>
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<tr>
<td>Insulin, ng/ml</td>
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<td>Leptin, ng/ml</td>
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Values are means ± SE, n = 8–10/group. Glucose (fasted), plasma free fatty acid (FFA) (fasted), insulin (fasted), and leptin (fed) levels of female and male mice after 15 wk of LFD or HFD feeding. *P < 0.05 vs. females LFD; **P < 0.05 vs. males LFD; ***P < 0.05 vs. females HFD. Factor interaction for insulin and leptin *p<sex*<diet<0.001; 2-way ANOVA (Bonferroni posttest).

Fig. 4. A: relative amounts of adipocytokine mRNA extracted from epicardial fat of HFD-fed mice at 25 wk. Data are shown as x-fold expression over levels in female epicardial fat. Values are displayed as means ± SE. *P < 0.05 (n = 5–8 mice/group, two-sided, unpaired t-test). B: vaspin serum levels. Displayed are mean vaspin serum concentrations (in pg/ml ± SE) from 25 wk HFD-fed male and female animals; P = not significant (n = 5 mice/group 2-sided, unpaired t-test).
potential mechanisms underlying the sex differences in HFD-induced LVH. Sexual dimorphisms were detected for leptin, adiponectin, and vaspin mRNA expression in epicardial fat. Leptin has been shown to induce a hypertrophic response in cardiomyocytes and may contribute to the present sex-dependent cardiac phenotype (24). Since HFD-fed animals showed similar serum leptin levels (Table 3), leptin is unlikely a key modulator of heart hypertrophy in our model. After 15 wk of HFD, only male animals showed significant insulinemia compared with other animals, which indicates that insulin, as previously reported, could also be considered as a potential coregulator of LVH in our model (17).

Although the role of adiponectin in the development of the pathological form of LVH is still elusive, adiponectin was shown to play a protective role in the development of pressure-induced cardiac hypertrophy, since adiponectin-deficient mice exhibit enhanced concentric hypertrophy and mortality after transverse aortic constriction (28). On the contrary, data published by O’Shea and collaborators (22) indicate that adiponectin deficiency did not influence the LVH in the transverse aortic constriction model in mice. Instead, the author suggested, that adiponectin rather plays a permissive role determining myocardial LV chamber remodeling and mitochondrial oxidative capacity of the heart.

The local adiponectin expression measured in male epicardial adipose tissue was higher in males compared with females, which is discrepant to its protective role. Thus it appears that in our model local adiponectin action does not play a major role in modulating sex-specific regulation of diet-induced LVH.

Recently, published work of Nishizawa and colleagues (21) indicates that adiponectin is negatively regulated by testosterone in vivo and in vitro. Since we did not measure plasma adiponectin levels, we cannot exclude that adiponectin affects sex-specific differences in the development of LVH observed in our model. On the other hand, a study published by Hecker and colleagues (11) showed that adiponectin does not play a crucial role during the development of LVH under HFD.

In the present study we investigated the role of the serine protease inhibitor vaspin in cardiac hypertrophy. Vaspin is a 47-kDa protein isolated from rat visceral adipose tissue and supposed to mediate insulin-sensitizing actions. In humans, vaspin plasma level and adipose tissue mRNA expression positively correlate with obesity, insulin resistance, and diabetes mellitus type 2 (12, 34). However, the molecular target of vaspin and underlying molecular mechanisms influencing insulin and glucose metabolism are still unknown. Here we show that vaspin expression was increased in male epicardial adipose tissue and induced cardiac fibroblast proliferation, a process
likely contributing to the sex-dependent development of LVH. Vaspin did not regulate cardiac myoblast hypertrophy. Since vaspin belongs to the family of serine protease inhibitors (12), one may hypothesize that a cardiac serine protease could serve as the molecular target of vaspin mediating its profibrotic actions. Future experiments are required to identify this target enzyme.

Interestingly, we could not detect any sex-dependent regulation of vaspin serum levels in our model. In line, vaspin was also not regulated in primary white adipocytes after stimulation with 17β-estradiol (data not shown). This indicates that sex-specific regulation of vaspin seems to be specific for the epicardial fat depot. This is in concordance with a clinical study by Youn and colleagues (34) showing a sex-dependent discrepancy between vaspin mRNA expression in different adipose tissue depots and circulating vaspin levels. In addition, these authors demonstrate an adipose tissue depot-specific expression pattern of vaspin. Thus it appears that the epicardial adipose tissue depot has a distinct adipocytokine expression profile different from circulating levels.

Induction of hypertrophic cardiomyopathy by HFD has been shown by various investigators (6, 23). Increased levels of long-chain fatty acids such as palmitic acid have been proposed as potential mediators inducing LVH, e.g., by inactivating forkhead transcription factor Foxo3a (6). Since Foxo3a is involved in the regulation of fibroblast proliferation (20), it might be a potential molecular intersection of vaspin and fatty acid signaling contributing to sex-dependent HFD-induced LVH.

In summary, the present study shows an augmented LVH response of male mice to HFD that may be linked to a sex-specific adipocytokine profile in epicardial adipose tissue. In addition, we identified vaspin as a potential paracrine profibrotic factor regulated in a sex-dependent manner in the epicardial adipose tissue and likely involved in the sexual dimorphism of HFD-induced LVH.

GRANTS

This study was supported by the Deutsche Forschungsgemeinschaft Grants DFG-GK 754-III (to C. Böhm); DFG-KI 712/5-1, KFO 218/1 (to A. Foryst-Ludwig); and DFG-GK 754-III, DFG-KI 712/3-2, DFG-KI 712/5-2 (FG 1054/2), DFG-KI 712/6-1 (KFO 218/1) (to U. Kintscher).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00593.2012 • www.ajpheart.org


