Sex differences in physiological cardiac hypertrophy are associated with exercise-mediated changes in energy substrate availability

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1Center for Cardiovascular Research, Institute of Pharmacology, Charité-Universitätsmedizin Berlin; 2Department for Nuclear Medicine, University Clinic Würzburg; 3Department of Nephrology/Intensive Care Medicine, Charité Campus Virchow-Klinikum, and Center for Cardiovascular Research, Charité-Universitätsmedizin Berlin; 4Center for Cardiovascular Research, Charité-Universitätsmedizin Berlin, and Institute of Gender in Medicine, Berlin; 5Department of Endocrinology, Diabetes and Nutrition, Charité-Universitätsmedizin Berlin; and 6Department of Cardiology, German Heart Institute, Berlin, Germany

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Foryst-Ludwig A, Kreissl MC, Sprang C, Thalke B, Böhm C, Benz V, Gürген D, Dragun D, Schubert C, Mai K, Stawowy P, Spranger J, Regitz-Zagrosek V, Unger T, Kintscher U. Sex differences in physiological cardiac hypertrophy are associated with exercise-mediated changes in energy substrate availability. Am J Physiol Heart Circ Physiol 301: H115–H122, 2011. First published April 8, 2011; doi:10.1152/ajpheart.01222.2010.—Exercise-induced cardiac hypertrophy has been recently identified to be regulated in a sex-specific manner. In parallel, women exhibit enhanced exercise-mediated lipolysis compared with men, which might be linked to cardiac responses. The aim of the present study was to assess if previously reported sex-dependent differences in the cardiac hypertrophic response during exercise are associated with differences in cardiac energy substrate availability/utilization. Female and male C57BL/6J mice were challenged with active treadmill running for 1.5 h/day (0.25 m/s) over 4 wk. Mice underwent cardiac and metabolic phenotyping including echocardiography, small-animal PET, peri-exercise indirect calorimetry, and analysis of adipose tissue (AT) lipolysis and cardiac gene expression. Female mice exhibited increased cardiac hypertrophic responses to exercise compared with male mice, measured by echocardiography [percent increase in left ventricular mass (LVM)]; female: 22.2 ± 0.8%, male: 9.0 ± 0.2%; P < 0.05]. This was associated with increased plasma free fatty acid (FFA) levels and augmented AT lipolysis in female mice after training, whereas FFA levels from male mice decreased. The respiratory quotient during exercise was significantly lower in female mice indicative for preferential utilization of fatty acids. In parallel, myocardial glucose uptake was reduced in female mice after exercise, analyzed by PET [injection dose (ID)/LVM [%ID/g]: 36.8 ± 3.5 female sedentary vs. 28.3 ± 4.3 female training; P < 0.05], whereas cardiac glucose uptake was unaltered after exercise in male counterparts. Cardiac genes involved in fatty acid uptake/oxidation in females were increased compared with male mice. Collectively, our data demonstrate that sex differences in exercise-induced cardiac hypertrophy are associated with changes in cardiac substrate availability and utilization.

cardiac glucose uptake; lipolysis; small animal positron emission topography

CARDIAC ADAPTATION TO SUSTAINED physical exercise results in left ventricular (LV) hypertrophy and increased cardiac output (3). This physiological form of LV hypertrophy differs substantially from pathological LV hypertrophy, which occurs in aortic stenosis or chronic arterial hypertension (34). In contrast to pathological LV hypertrophy, physiological hypertrophy is characterized by cardiac myocyte enlargement leading to eccentric hypertrophy and does not result in heart failure (7). The development of exercise-mediated cardiomyopathy in mice is regulated in a sex-specific manner. Female mice exhibit an increased cardiac hypertrophic response in both voluntary and treadmill protocols compared with male mice (5, 23). In addition, female C57BL/6J mice show an increased exercise capacity compared with male mice initially suggesting that sexual dimorphisms in cardiac responses are mainly mediated by differences in running distance (5, 23). However, when cardiac hypertrophic responses were normalized to running distance, female mice still showed enhanced LV hypertrophy likely indicating a true sexual dimorphic cardiac response (23). The underlying mechanisms of these sex-mediated differences in cardiac hypertrophy are incompletely understood. Konhilas et al. (23) investigated multiple signaling molecules involved in the development of cardiac hypertrophy including Cu2+/calmodulin-dependent protein kinase (CaMK), myocyte enhancer factor-2 (MEF-2), mitogen-activated protein kinase (MAPK-ERK1/2), Akt, and glycogen synthase kinase-3β (GSK-3β). This analysis revealed an increased proportional increase of CaMK-activity in trained female hearts compared with males and a persistent phosphorylation of GSK-3β indicating an inactivation of this antihypertrophic factor. All other signal molecules did not show a sex-dependent regulation during exercise.

In addition to myocardial signaling, alterations in cardiac energy substrate metabolism have been implicated in the pathogenesis of LV hypertrophy and heart failure (31). An important adaptive process in the diseased hypertrophied heart is an increase in glucose utilization and a decrease in fatty acid oxidation (31). This is in contrast to metabolic processes during the development of physiological LV hypertrophy. Myocardial substrate utilization is highly dependent on systemic and local substrate availability. During physical training, major changes occur in substrate availability that may have important consequences for the development of exercise-induced physiological LV hypertrophy (22). Enhanced β-adrenergic receptor stimulation during exercise results in increased
lipolytic rate followed by elevated free fatty acid (FFA) plasma levels (13, 22). These processes establish fatty acids as the major cardiac energy substrate during exercise. Mobilization of FFAs from adipose tissue is the first committed step in fatty acid metabolism, and it depends on the rate of adipose tissue lipolysis (38). Fatty acids are deposited in adipose tissue as triacylglycerol (TG) and become hydrolyzed in periods of increased energy demand, e.g., during exercise (38). Women show higher adipose tissue lipolysis than men during exercise (27). The hydrolysis of TG in fat tissue is catalyzed by adipose tissue lipases. Hormone-sensitive lipase (HSL) has been suggested to be the rate-limiting enzyme for diacylglycerol hydrolysis (15). On the other hand, the adipose triglyceride lipase (ATGL) removes the first fatty acid from the TG molecule generating FFA and deoxyglucose (39). HSL and ATGL have been described as the major enzymes contributing to TG breakdown in adipose tissue and other tissues. Furthermore, these enzymes are crucial mediators of TG mobilization during exercise (1, 18). Whether enhanced exercise-induced lipolysis in women is associated with a sexual dimorphic regulation of lipolysis is currently unknown.

In the present study, we hypothesize that sexual dimorphisms in systemic energy substrate metabolism are closely connected to sex differences in physiological LV hypertrophy.

METHODS

Ethical approval. All animal procedures were performed in accordance with the guidelines of the Charité Medical University Berlin and were approved by the Landesarztes für Gesundheit und Soziales (LaGeSo, Berlin, Germany) for the use of laboratory animals and followed the current version of the German Law on the Protection of Animals.

Animals. Five-week-old female and male C57Bl/6J mice were housed in a temperature-controlled (25°C) facility with a 12-h light-dark cycle. Mice were fed ad libitum with standard diet (Smiff, Soest, Germany: 58 kcal from carbohydrates, 33 kcal from protein, and 9 kcal from fat), and body weight development was monitored throughout the experiment. Initially, mice were randomized to treadmill-trained group (run: n = 18 male and n = 18 female mice) and control sedentary animals (sed: n = 10 male and n = 10 female mice). At the end of the training regime, all mice (run + sed) underwent echocardiographic analysis. Blood samples were collected by retroorbital venous puncture under short isoflurane anesthesia before/after 45 min of training in fed mice for analysis of plasma glucose (assessed with glucometer: Precision Xtra, Abbott, Abbott Park, IL), lactate (assessed with lactate analyzer: Lactate Scout), plasma TGs (DiaSys, Diagnostic Systems), and plasma FFA (Wako Chemicals). Body composition was determined by nuclear magnetic resonance imaging (Bruker’s Minispec M Q10). Subsequently, the respiratory quotient (RQ) during exercise was determined in run mice using indirect calorimetric analysis combined with treadmill training (TSE Systems). This analysis was performed during one individual training episode after 4 wk of training. Finally, myocardial substrate utilization study was performed in all groups of mice (n = 6 per group) using small animal PET. After a 24-h recovery phase, mice were killed and organs were dissected, shock-frozen in liquid nitrogen, and stored for expression analysis. For ex vivo lipolysis assays, a subgroup of run mice (n = 4) were killed directly after training. To avoid circadian variation all mice were killed at the same time of the day (early morning hours).

Training and calorigametry. Run mice were adapted to treadmill training (treadmill from TSE Systems) by gradual increase of the training intensity (0.05–0.25 m/s, 7° slope, 15–90 min·day⁻¹·mouse⁻¹, for 3 wk). After the adaptation phase mice were trained over 4 wk using the following protocol: 0.25 m/s, 7° slope, 90 min·day⁻¹·mouse⁻¹. To avoid differences in nutritional status of the mice, all animals were trained in the fed state. Training was continuously monitored from one investigator, and mice were manually replaced when stepped from the treadmill lane. For RQ measurements, mice were placed in treadmills with integrated custom-made calorimetry system (CaloTreadmill; TSE Systems). The calorimetry system is an open-circuit system that determines O₂ consumption, CO₂ production, and RQ measurement at the basal state (without training) and during the treadmill training. All parameters were measured continuously.

Echocardiography. Echocardiography was performed after the 4-wk training period using a standardized protocol as previously described (11) and developed in accordance with established standards in human echocardiography. Mice were anesthetized with isoflurane and body temperature was maintained using a heating pad at 37°C. The use of the certain anesthetics during the procedure could potentially influence the absolute values for echocardiographic parameters (12, 30). A Vevo 770 High-Resolution Imaging System (VisualSonics, Toronto, ON, Canada) with a RMV 707 Scanhead (VisualSonics) was used for analysis.

Small animal PET. All animals had free access to water and chow before the study; blood glucose levels were monitored using blood samples drawn from the tail (Freestyle mini; Abbott). To assess glucose metabolism, the animals were injected with ~7 MBq of [18F]-2-fluorodeoxyglucose (18F-FDG) and imaged for 10 min after an awake uptake period of 50 min using a dedicated small animal PET (Inveon dedicated PET; Siemens, Erlangen, Germany). To evaluate fatty acid metabolism, ~2 MBq of [18F]-fluoro-4-thia-palmitate (18F-FTP) were injected; the 10-min data acquisition was started after a 30 min of awake uptake period (6). For anesthesia, 1.5% isoflurane in 50% air and 50% oxygen was applied, and the animals were allowed to recover for 24 h before both PET studies.

Data were reconstructed using 3D-OSEM, corrected for decay and injected dose. Image analysis was performed using AMIDE (26). Volumes of interest were assigned to the heart. Same size volumes of interest were used for all mice; for the heart volumes of interest, a size was chosen to include the whole myocardium (231 mm³). To obtain the percent injected dose per gram (%ID/g), the measured 18F-FDG uptake in the whole heart was normalized by the left ventricular mass (LVM) obtained from the echocardiographic analysis, described above.

RNA and protein analysis. Total RNA from LV and gonadal adipose tissue was isolated using Qiazol and RNeasy Micro kit (Qiagen), according to the manufacturer’s instruction. For real-time PCR analysis, RNA samples were DNase digested (Qiagen); reverse transcribed using reverse transcriptase, RNAsin, and dNTPs (Promega), according to the manufacturer’s instructions; and used in quantitative PCR reactions in the presence of a fluorescent dye (Sybrgreen, Life Sciences). Relative abundance of mRNA was calculated after normalization to 18S ribosomal. The primer sequences used for the measurements are available on demand.

For Western blot analysis, frozen LV heart tissue was lysed as described previously (14). Briefly, tissue samples were lysed using 1.4-mm ceramic lysis matrix (Precellys24; Peqlab, Erlangen, Germany) in lysis buffer [150 mM NaCl, 50 mM Tris·HCl pH 7.5, 1 mM EDTA, 1 mM EGTA, 1% Igepal CA-630, 1 mM sodium vanadate, 2 mM sodium pyrophosphate, 10 mM sodium fluoride, 10 mM β-glycerophosphate, 0.1% SDS, and complete protease inhibitor cocktail (Complete Mini; Roche Diagnostics)]. Homogenization was achieved in a tissue disruptor (FastPrep24; MPBiomedicals, Solon, OH) for 2 × 20 s. Lysates were analyzed by immunoblotting using antibodies raised against pS473-Akt and total-Akt (4060 and 9272 from Cell Signaling Technologies) and a secondary horseradish-conjugated antibodies (Jackson ImmunoResearch). For detection, enhanced chemiluminescent reagents (ECL kit; Thermo Scientific) were used.
Table 1. Basic metabolic characterization

<table>
<thead>
<tr>
<th></th>
<th>Males Sed</th>
<th>Males Run</th>
<th>Females Sed</th>
<th>Females Run</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>179.8 ± 13.1</td>
<td>190.7 ± 28.2</td>
<td>178.7 ± 18.1</td>
<td>182.0 ± 24.4</td>
</tr>
<tr>
<td>Lactate, mg/dl</td>
<td>3.7 ± 1.4</td>
<td>3.3 ± 0.9</td>
<td>3.2 ± 1.9</td>
<td>2.8 ± 1.0</td>
</tr>
<tr>
<td>BW, g</td>
<td>28.8 ± 0.6</td>
<td>27.7 ± 0.7</td>
<td>22.7 ± 0.1</td>
<td>22.9 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. All measurements were performed after 4 wk of training. BW, body weight. *P < 0.001 vs. males sedentary (sed); †P < 0.001 vs. males run [n = 6–10; two-way ANOVA (Bonferroni posttest)].

Ex vivo lipolysis assay in gonadal adipose tissue explants. The ex vivo lipolysis assay was adapted from previously published studies (2). Gonadal fat pads were surgically removed from run mice directly after training (n = 4/group) and washed with ice-cold PBS. Adipose tissue excised pads (3 mm × 3 mm, n = 3/mouse) were incubated in 140 μl DMEM (Invitrogen) containing 2% fatty acid free serum albumin (BSA, Sigma) for 2 h at 37 °C. Afterwards, FFA content was quantified using the kit, and released glucose was quantified calorimetrically using the appropriate standard curve.

Table 2. Cardiac phenotyping

<table>
<thead>
<tr>
<th></th>
<th>Males Sed</th>
<th>Males Run</th>
<th>Females Sed</th>
<th>Females Run</th>
</tr>
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<tbody>
<tr>
<td>HR, beats/min</td>
<td>428.4 ± 14.2</td>
<td>420 ± 27.7</td>
<td>431.9 ± 10.9</td>
<td>453.2 ± 9.8</td>
</tr>
<tr>
<td>EF, %</td>
<td>45.7 ± 1.6</td>
<td>46.2 ± 2.0</td>
<td>48.8 ± 1.2</td>
<td>49.5 ± 1.5</td>
</tr>
<tr>
<td>PWd, mm</td>
<td>0.61 ± 0.09</td>
<td>0.65 ± 0.008</td>
<td>0.59 ± 0.003</td>
<td>0.64 ± 0.010</td>
</tr>
<tr>
<td>IVSd, mm</td>
<td>0.63 ± 0.008</td>
<td>0.64 ± 0.004</td>
<td>0.60 ± 0.006</td>
<td>0.64 ± 0.006</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>4.48 ± 0.09</td>
<td>4.58 ± 0.07</td>
<td>4.69 ± 0.06</td>
<td>4.43 ± 0.09</td>
</tr>
<tr>
<td>LVM, mg</td>
<td>102.6 ± 3.8</td>
<td>111.9 ± 2.9</td>
<td>82.4 ± 2.3</td>
<td>100.7 ± 3.7</td>
</tr>
<tr>
<td>LVM/BW, mg/g</td>
<td>3.57 ± 0.10</td>
<td>3.92 ± 0.11</td>
<td>3.63 ± 0.10</td>
<td>4.42 ± 0.18</td>
</tr>
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Values are means ± SE. Echocardiographic parameters performed after 4 wk of training. HR, heart rate; EF, ejection fraction; PWd, posterior wall thickness during diastole; IVSd, septum thickness during diastole; LVIDd, LV internal diameter during diastole; LVM, left ventricular mass. *P < 0.01 vs. males sed; †P < 0.001 vs. females sed; ‡P < 0.001 vs. males sed; §P < 0.05 vs. males run; ††P < 0.01 vs. females sed [n = 6; two-way ANOVA (Bonferroni posttest)].

body weight (BW) compared with female mice, but BW was not significantly affected by training in both sexes (Table 1).

Echocardiography. To evaluate whether a sex-dependent adaptive cardiac response to forced treadmill running occurs, we performed echocardiographic analysis. The results are outlined in Table 2 and Fig. 1. LVM was higher in male C57Bl/6J mice compared with females (Table 2). LVM after exercise (run) did only significantly increase in female mice compared with sedentary (sed) control groups (Table 2). LVM normalized to BW exhibited a stronger exercise-induced increase in female mice compared with male mice (Table 2). To better compare the magnitude of hypertrophy, we calculated the percent increase in LVM as previously described (23).

As depicted in Fig. 1, the percent increase in LVM was significantly higher in female mice compared with males. Together with the data in Table 2, these data corroborate a stronger adaptive response in females including increased septum thickness during diastole and posterior wall thickness during diastole in female run mice compared with female sed mice. Male mice showed only enhanced posterior wall thickness during diastole thickness in male run mice (Table 2). In consonance, heart weight-to-tibia length ratio was increased in females run vs. sed but not in males [heart weight-to-tibia length ratio (in mg/mm): females sed: 7.7 ± 0.5 vs. females run: 9.9 ± 0.7 (P < 0.01); males sed: 9.3 ± 0.6 vs. males run: 10.2 ± 0.8 (n.s.)]. To support our echocardiographic data, we additionally analyzed cardiac Akt signaling, a typical molecular marker for physiological myocardial hypertrophy (21). Akt has been shown to be hyperphosphorylated in physiologically hypertrophied hearts (21). As depicted in Supplemental Fig. S1A (Supplemental Material for this article is available online at the
Am J Physiol Heart Circ Physiol website), the phosphorylation status of cardiac Akt was increased in female run mice compared with female sed mice, whereas this regulation was absent in male mice. In summary, our echocardiographic analysis indicates an enhanced adaptive hypertrophic response to exercise in female hearts compared with males.

**Exercise-mediated metabolic changes.** To determine exercise-mediated changes in systemic energy substrate availability and utilization, we studied metabolic parameters directly before, during, and after a training session at the end of the 4-wk training period. As presented in Fig. 2A, plasma glucose levels did not markedly change in both sexes after exercise. In contrast, plasma FFAs significantly changed with exercise. A significant sex-dependent difference was documented, with a decrease of FFA levels in male mice after exercise and an increase in female mice [(in mmol/l) female: pre: 0.59 ± 0.06 vs. post: 0.7 ± 0.04; male pre: 0.96 ± 0.08 vs. post: 0.7 ± 0.07; *P < 0.05 female vs. male; *P < 0.05 female pre vs. post; Fig. 2B]. In sed mice FFA plasma levels were higher in males than in females (Supplemental Fig. S2A), a similar pattern as in run mice before the training episode (Fig. 2B). Plasma triglycerides showed the same pattern of regulation, but significant sex-specific differences were only detected in run mice before the training episode (Fig. 2C; Supplemental Fig. S2A). Next, we studied whole body energy substrate utilization/oxidation by peri-exercise indirect calorimetry using custom-made treadmills integrated into an open-circuit calorimetry system (Fig. 2D). We detected significant lower RQ values during exercise indicative for augmented lipid oxidation in female mice compared with their male counterparts (Fig. 2D).

To further determine a potential mechanism for sex-specific metabolic responses to exercise, we studied mRNA expression of two major adipose tissue lipases, ATGL and HSL, in gonadal white adipose tissue from female and male mice after exercise. As depicted in Fig. 2E, ATGL expression was increased in female mice compared with male mice, whereas HSL expression did not show any sex-specific regulation.

Fig. 2. Exercise-mediated metabolic changes. Change in plasma glucose (A), free fatty acid (FFA; B), and triacylglycerol (TG; C) levels of trained female and male mice before (pre) and directly after (post) a training episode; *P < 0.05 vs. pre/post difference in male mice. **P < 0.05 vs. males [n = 10–18; two-way ANOVA with repeated measures (Bonferroni posttest)]. D: respiratory quotient (RQ) from trained mice during exercise. RQ was calculated as the ratio between CO₂ produced (V˙CO₂) and O₂ consumed (V˙O₂) using indirect calorimetry integrated into the treadmill system (CaloTreadmill; TSE System), described in details under METHODS. *P < 0.05 vs. males [n = 8; two-way ANOVA with repeated measures (Bonferroni posttest)]. E: analysis of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) mRNA expression levels in gonadal adipose tissue from trained mice, as a marker of fat-tissue specific lipolytic activity. Real-time quantitative RT-PCR studies were carried out using total RNA isolated from gonadal fat. Data are presented as x-fold over males run. For details, see METHODS. *P < 0.05 vs. males (n = 6; unpaired t-test). F: ex vivo lipolysis assay in murine gonadal adipose tissue explants. Gonadal fat pads were isolated from run mice directly after training (n = 4/group). FFA-release assay was performed using adipose tissue pads (n = 3/mouse) as described in METHODS. *P < 0.05 vs. males (unpaired t-test).
and male mice after exercise (Fig. 2F). In accordance, female mice showed higher rates of adipose tissue lipolysis after exercise compared with male mice.

In summary, the present data demonstrate that female mice exhibit an increase of circulating FFAs during exercise associated with enhanced peri-exercise lipid oxidation, increased ATGL expression, and greater rates of adipose tissue lipolysis.

**Cardiac metabolism: PET and gene expression.** Small animal PET was performed in sed and run mice (after 4 wk of forced treadmill running) in a fed state. We performed FTP- and FDG-PET to assess cardiac FA and glucose uptake. In accordance with previously published studies (10), we were not able to detect major cardiac FA uptake in male/female mice under resting/fed conditions (data not shown). Therefore, subsequent analysis was focused on myocardial FDG uptake. In sed mice, glucose uptake was higher in female than in male hearts (Fig. 3, A and B). Male mice challenged with 4 wk of training did not show any significant differences in cardiac glucose uptake compared with male sed controls (Fig. 3, A and B). In contrast, trained female mice showed significantly reduced cardiac glucose uptake compared with resting mice (Fig. 3, A and B).

Since analysis of cardiac FA uptake was not applicable in PET analysis, we indirectly assessed the activity of the FA oxidative pathway by studying cardiac mRNA expression of enzymes involved in mitochondrial fatty acid oxidation and triglyceride catabolism, such as carnitine palmitoyl transferase 1β, acyl-CoA synthetase long-chain family member 1, and peroxisome proliferator-activated receptor-α, and fatty acid transporter CD36 was evaluated (Fig. 4A). In female mice, LV expression of these genes was significantly higher than in male mice after exercise suggesting a training-mediated stimulation of cardiac FA metabolism in female mice. In sed mice only CD36 expression was higher in female hearts than in male hearts (Supplemental Fig. S2C). In addition, the expression of genes involved in cardiac glucose metabolism including glucose transporter (GLUT)-1 and GLUT-4, and pyruvate dehydrogenase kinase 4 was studied (Fig. 4B). Cardiac GLUT-1 and GLUT-4 expression was lower in female compared with male mice after exercise. In sed mice, these genes were not sex

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**Fig. 3. Cardiac metabolism: PET.** A: representative images of the myocardial glucose uptake measured in the coronal layer (top) or transversal layer (bottom) using [18F]-2-fluorodeoxyglucose (18F-FDG) small animal PET analysis. B: summarized results of 18F-FDG PET, showed as injection dose relative to LVM. *P < 0.05 vs. females sedentary (sed). °P < 0.001 vs. males sed. #P < 0.05 vs. males run [n = 6; 2-way ANOVA (Bonferroni posttest)].
FA preference as the cardiac energy substrate during exercise
expression of genes involved cardiac FA oxidation, suggesting an uptake in trained female mice associated with increased exercise-induced LV hypertrophy in female mice compared with male mice. By using a forced treadmill running protocol, continuously monitored by investigators, we tried to assure identical running distance in both sexes thereby identifying sex-mediated LV changes independent from differences in exercise performance. Both sexes ran identical distances; however, when running distance was normalized to lean mass, female mice exhibited a higher level of exercise per grams lean mass compared with male mice. Active muscle mass and training intensity are related to the magnitude of pressor responses during exercise (19, 25). This may result in distinct cardiac hypertrophic responses. Thus we cannot exclude that sex-specific differences in running distance/lean mass contributed to the development of cardiac hypertrophy in our model. Furthermore, in the present study the adaptive training phase in female/male mice started at the age of 5 wk with full training from 8 to 12 wk of age. Start of the initial training while mice were in their growth phase may have influenced the observed differences. Previous studies (5, 23) documented similar sex-specific differences in exercise-induced cardiac hypertrophy using 12-wk-old and 10- to 11-wk-old mice, suggesting that these processes observed in our study are age independently regulated. However, an interaction between cardiac growth and training cannot be completely ruled out in the present study.

Studies in humans comparing exercise-mediated cardiac hypertrophy in both sexes are limited. Scharhag et al. (33) studied LVM by MRI in male endurance athletes and untrained control. LVM was significantly increased in male endurance athletes by 36 ± 14% compared with untrained control subjects (33). Sex differences in LVM were investigated with MRI in young adult elite athletes and age- and sex-matched sedentary controls (28). In contrast to our results, female and male athletes exhibited similar increases in LVM and LVM indexes compared with their sedentary controls (28). However, despite a comparable training duration (hours/week and years at elite level) in female and male athletes, no detailed information about training intensity was provided (28). Putative sex differences in training intensity likely influence exercise-induced cardiac remodeling. In our experiment, both sexes were challenged with an identical training intensity, explaining, at least in part, the discrepancy between an observational human study and the present experiments in rodents. To our knowledge, no longitudinal clinical study determining exercise-mediated cardiac hypertrophy in a sex-specific manner including identical training protocols has been published so far.

Potential mechanisms underlying this sexual dimorphism in physiological cardiac hypertrophy have been recently proposed focusing on myocardial signaling. Furthermore, previous studies (11, 35) have demonstrated an important role of sex hormones as direct modifiers of hypertrophic responses in the...
myocardium. However, these studies did mainly investigate pathological and not physiological myocardial hypertrophy. In the present study, we focused on exercise-dependent alterations in systemic and cardiac energy substrate metabolism as a potential mechanism for sexual dimorphisms in physiological cardiac hypertrophy. Preservation or augmentation of cardiac FA utilization as the primary fuel source during sustained physical training has been suggested as one of the important mechanisms distinguishing adaptive from maladaptive LV hypertrophy (7). Cardiac expression profiling studies in physiological vs. pathological hypertrophy revealed increased expression of genes involved in FA metabolism such as CD36 in exercise-induced hypertrophy (37). Also, our results suggest that female mice, in response to exercise, have higher rates of cardiac lipid metabolism, as indicated by the transcriptional upregulation of genes involved in fatty acid uptake and mitochondrial fatty acid oxidation. Comparisons of male and female individuals during exercise with regard to their lipid metabolism revealed that women have higher rates of lipid oxidation compared with men resulting from enhanced adrenergic lipolysis and increased plasma FFA levels (4, 16, 27). Parallel to a stronger hypertrophic response, female mice exhibited increased postexercise plasma FFA levels and lower RQ values, indicative for enhanced lipid oxidation, as shown by Carter et al. (4) in humans. These sex differences may result from enhanced adipose tissue lipolysis in trained female mice compared with males and increased expression of ATGL, a crucial lipase of triglyceride breakdown in adipose tissue.

Our PET studies revealed a significantly higher cardiac glucose uptake in female sed hearts compared with male hearts. The underlying mechanism of this sexual dimorphism in sedentary mice is unclear. Previously, Duvernoy et al. (8) demonstrated a significantly higher resting myocardial blood flow in women compared with men. Since myocardial blood flow positively correlates to cardiac FDG uptake (20), enhanced female myocardial blood flow at rest may contribute to sex-dependent differences in cardiac glucose uptake in our study.

In addition, we could show that simultaneous cardiac glucose uptake decreases in female mice after exercise, whereby no differences in glucose uptake were observed in male mice. A cross talk between nutrient utilization has been appreciated for many years (29). Based on these findings, various molecular mechanisms have recently identified how increased fatty acid availability and/or oxidation negatively interferes with glucose uptake/metabolism (17). In this context, FAs have also been shown to inhibit glucose metabolism in cardiac myocytes by blocking 2-deoxyglucose uptake (9). These data provide an explanation for our observation that exercise-induced increases in plasma FFA level are closely linked to a decrease in cardiac glucose uptake in female mice. A direct analysis of cardiac FA uptake would strengthen our hypothesis. Unfortunately, using the current PET protocol, we were not able to detect any major cardiac FA uptake in both sexes. These data are in accordance with previously published studies (10) showing very low levels of cardiac FA uptake in resting mice. To detect a reasonable cardiac FA uptake, it appears that mice require a defined adrenergic stimulus for the intermittent increase in circulating FA levels providing the required cardiac energy substrate. Since exercise during small animal PET is not applicable, we are currently establishing a protocol with dobutamine injection during PET analysis of FA uptake, as previously shown for FDG (24).

Along this line, Soto et al. (36) recently investigated these processes in humans. With the use of cardiac PET analysis, myocardial glucose and fatty acid utilization was studied in 12 healthy older individuals (6 men/6 women) before and after an 11-mo endurance exercise training period (36). In consonance with our data, the authors found increased posttraining plasma FFA levels in women compared with men. More importantly, this was associated with increased dobutamine-induced cardiac fatty acid utilization posttraining in females but not in males, corroborating the predominance of lipids as the “female” cardiac fuel during exercise.

Conclusions. In summary, our data show that sex differences in exercise-induced cardiac hypertrophy are associated with changes in cardiac substrate availability and utilization. These processes provide an additional new mechanism for sex differences in physiological cardiac hypertrophy.

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DISCLOSURES

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

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