Expression of lipogenic genes is upregulated in the heart with exercise training-induced but not pressure overload-induced left ventricular hypertrophy

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Dobrzyn P, Pyrkowska A, Duda MK, Bednarski T, Maczewski M, Langfort J, Dobrzyn A. Expression of lipogenic genes is upregulated in the heart with exercise training-induced but not pressure overload-induced left ventricular hypertrophy. Am J Physiol Endocrinol Metab 304: E1348–E1358, 2013. First published April 30, 2013; doi:10.1152/ajpendo.00603.2012.—Cardiac hypertrophy is accompanied by molecular remodeling that affects different cellular pathways, including fatty acid (FA) utilization. In the present study, we show that cardiac lipid metabolism is differentially regulated in response to physiological (endurance training) and pathological [abdominal aortic banding (AAB)] hypertrophic stimuli. Physiological hypertrophy was accompanied by an increased expression of lipogenic genes and the activation of sterol regulatory element-binding protein-1c and Akt signaling. Additionally, FA oxidation pathways regulated by AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-α (PPARα) were induced in trained hearts. Cardiac lipid content was not changed by physiological stimulation, underlining balanced lipid utilization in the trained heart. Moreover, pathological hypertrophy induced the AMPK-regulated oxidative pathway, whereas PPARα and expression of its downstream targets, i.e., acyl-CoA oxidase and carnitine palmitoyltransferase I, were not affected by AAB. In contrast, pathological hypertrophy leads to cardiac triglyceride (TG) and diacylglycerol (DAG) accumulation, although the expression of lipogenic genes and the levels of FA transport proteins (CD36 and FATP) were not changed or reduced compared with the sham group. A possible explanation for this phenomenon is a decrease in lipolysis, as evidenced by the increased content of adipose triglyceride lipase inhibitor G0S2, the increased phosphorylation of hormone-sensitive lipase at Ser565, and the decreased protein levels of acyl-CoA dehydrogenase and carnitine palmitoyltransferase I (CPT I), which in due course will affect cardiac function and ultimately contribute to the transition from compensatory hypertrophy to cardiac failure (70). Molecular remodeling in the heart caused by hypertrophy differs between physiological and pathological stimuli. The physiological cardiac hypertrophy caused by endurance training shows enhancement of cardiac function at rest and during exercise and is not a risk factor for heart failure (19, 29). The adaptations include increases in cardiac mass and dimension, maximum oxygen consumption, and coronary blood flow (35). Additionally, exercise results in a balanced growth of cardiomyocytes with a normal myofibril to mitochondrial ratio (52, 71). Conversely, the hypertrophy observed in different pathological settings is accompanied by cardiac dysfunction or increased morbidity (61). The failing heart is characterized by alterations in energy metabolism, including mitochondrial dysfunction and a reduction in the fatty acid (FA) oxidation rate, which is compensated partially by an increase in glucose utilization (52, 69).

Both physiological and pathological cardiac hypertrophy cause changes in lipid metabolism in the heart, although little is known about the underlying molecular changes. Studies primarily concerned the FA oxidation pathway and presented the downregulation of genes involved in FA transport and oxidation in pathological hypertrophy, whereas upregulation of several of these genes was observed in adaptive hypertrophy (63). The decreased expression of genes involved in FA oxidation that is observed in pathological hypertrophy is believed to be due to a decline in the activity of peroxisome proliferator-activated receptor-α (PPARα), the transcription factor that plays a crucial role in the transcriptional regulation of genes involved in FA metabolism (21, 30, 52, 69). Moreover, expression of PPARα and its downstream targets, i.e., medium-chain acyl-CoA dehydrogenase and carnitine palmitoyltransferase I (CPT I), was upregulated after endurance training (52). Therefore, it has been proposed that the higher rates of FA oxidation observed in trained hearts may partially prevent the accumulation of fatty acids and activated fatty acids to limit their toxic effects in the cytosol and mitochondria (6).

However, it remains to be established whether physiological and pathological left ventricle (LV) hypertrophy affects the expression of genes involved in lipogenesis and in turn affects cardiac lipid content. Many studies underline the important role of lipogenic genes in proper heart function. It has been shown that cardiac-specific knockdown of PPARγ (17) and acyl-CoA synthase 1 (18) induces cardiac hypertrophy. It has also been shown that transgenic overexpression of FA transport protein 1 (FATP1) in the heart causes lipotoxic cardiomyopathy (9). Additionally, expression of FA synthase (FAS), the enzyme that catalyzes de novo FA synthesis, was induced in the hypertrophied hearts of heart-specific acyl-CoA synthase-overexpressing mice, in the hearts of heart-specific TNF receptor-associated factor 2 transgenic mice with inflammation-
mediated heart failure, and in the hearts of humans with end-stage cardiomyopathy (50). These results suggest that FAS is increased in both mouse and human heart failure. Interestingly, mice with a knockout of FAS in the myocardium develop normally and manifest normal resting heart function (50). However, most of these knockout mice die within 1 h of transverse aortic constriction, which likely due to arrhythmia caused by calmodulin-dependent protein kinase II hyperactivation (50). Another important lipogenic enzyme involved in cardiac substrate utilization and function is stearoyl-CoA desaturase (SCD). The lack of SCD1 expression decreases FA uptake and oxidation and increases glucose transport and oxidation in the heart (16). Disruption of the SCD1 gene improves the cardiac function in obese leptin-deficient ob/ob mice by correcting the systolic and diastolic dysfunction (14). The improvement is associated with the reduced expression of the genes involved in FA transport and lipid synthesis within the heart in addition to the reduction of the cardiac free fatty acid (FFA), diacylglycerol (DAG), triglyceride (TG), and ceramide levels (14). Taken together, all of these data show that although the myocardium has a low capacity for de novo lipogenesis, lipogenic genes and lipids play significant roles in the control of cardiac metabolism and remodeling.

Therefore, the main goal of this study was to determine how cardiac hypertrophy that is induced by pressure overload or endurance training influences lipid metabolism in the myocardium. The obtained results indicate that expression of lipogenic genes and sterol regulatory element-binding protein-1c (SREBP-1c) pathway was upregulated in exercise-induced hypertrophy. Increased lipogenesis was accompanied by the activation of FA oxidation pathways regulated by AMP-activated protein kinase (AMPK) and PPARα. Intracellular DAG, TG, FFA, and phospholipid (PL) levels were not changed in trained compared with untrained hearts. In contrast, in the heart with abdominal aortic banding (AAB)-induced hypertrophy, we found an increased accumulation of intracellular TG and DAG, although the expression of lipogenic genes and the level of FATPs were not augmented. Cardiac steatosis in AAB-induced hypertrophy was associated with decreased lipolysis, as evidenced by increased content of adipose triglyceride lipase (ATGL) inhibitor G0S2, increased hormone-sensitive lipase (HSL) phosphorylation at Ser565, and decreased DAG lipase (DAGLα) and DAGLβ protein levels.

MATERIALS AND METHODS

Experimental animals. For a physiological stress-induced cardiac hypertrophy model, 11-wk-old male Wistar rats were randomly assigned to either a treadmill running program (n = 6) or served as sedentary controls (n = 6). The animals had free access to food and water. Rats to be exercise trained were subject to running exercise 5 days/wk for 6 wk beginning at a treadmill speed of 16 m/min (0° inclination). The running speed was increased by 4 m·min⁻¹·wk⁻¹ over the first 4 wk and then maintained at 28 m·min for the remaining 2 wk of the endurance training. The running time was 40 min/day over the first 4 wk and was subsequently increased daily by 5 min to reach 60 min/day (15). The endurance-trained rats were anesthetized with 1.5–2.0% isoflurane and euthanized 48 h after the last exercise session. The heart muscle was excised, and the LV was frozen in liquid nitrogen and stored at −80°C.

For a pathologically induced cardiac hypertrophy model, 8-wk-old male Wistar rats were anesthetized with 1.5–2.5% isoflurane by mask inhalation. A blunt (20-gauge) needle was placed along the aorta, and both the aorta and the needle were tied with a 3-0 silk thread. The needle was removed, resulting in a diameter of the aortic lumen that was determined by the diameter of the needle. Sham surgery animals (n = 6) were subjected to an identical procedure without the aortic banding. Nine weeks after surgery, rats were anesthetized with 1.5–2.0% isoflurane. The LV was quickly removed, weighed, freeze-clamped and stored at −80°C. The experiments were approved by the Ethical Committees for Animal Experiments at the Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland, and at the Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland.

Materials. SCD1, CPTI, αβ-hydrolase domain containing 5 (CGI-58), GAPDH, putative lymphocyte G5/G1, switch protein 2 (G5S2), SREBP-1c, fatty acid translocase/CD36 (CD36), FABP1, PPARα, and β-actin antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). ATGL, AMPK, phosphorylated AMPK, Akt, phosphorylated Akt Ser473, HSL, phosphorylated HSL Ser565, and phosphorylated HSL Ser565 antibodies were obtained from Cell Signaling Technology, (Hertfordshire, UK), whereas phosphorylated acetyl-CoA carboxylase (ACC) antibody was obtained from Upstate Biotechnology (New York, NY). DAGLα and DAGLβ antibodies were obtained from Abcam (Cambridge, UK). Horseradish peroxidase-conjugated streptavidin was obtained from Pierce (Rockford, IL). Other chemicals were purchased from Sigma (St. Louis, MO). Insulin level was measured by using a rat/mouse insulin ELISA kit (Millipore, Billerica, MA).

Cardiovascular measurements. LV function was evaluated using Mylab25 (Esaote) with a 13-MHz linear array transducer. Each rat was examined at baseline and 6 wk after endurance training or 8 wk after the surgery. Under light anesthesia (1.5–2.0% isoflurane by mask inhalation) LV end-diastolic and end-systolic diameters, in addition to wall thickness, were determined from the short-axis view at the midpapillary level. LV end-diastolic and end-systolic diameters were planimetered from the parastral long-axis view. LV ejection fraction (EF%) was calculated as: EF (%) = (end-diastolic volume − end-systolic volume)/end-diastolic volume. Fractional shortening was calculated as: (LV diameter in diastole − LV diameter in systole)/LV diameter in diastole.

Isolation and analysis of RNA. Total RNA was isolated from hearts using TRIzol reagent (Invitrogen, Carlsbad, CA). DNase-treated RNA was reverse transcribed with SuperScript III (Invitrogen), and real-time quantitative PCR was performed on an ABI Prism 7500 Fast Instrument. SYBR green was used for the detection and quantification of genes that were expressed as mRNA, and the level was normalized to actin using the ∆∆CT method. The relative abundance of SCD1, SCD2, SREBP-1c, PPARα, acyl-CoA oxidase (ACO), CPTI, acyl-CoA synthase 1 (ACS), FABP1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was measured. Primer sequences are available on request.

Measurement of lipids. Lipids were extracted by the method of Bligh and Dyer (5) and measured as described previously (12). Briefly, the lipids were separated by thin-layer chromatography on silica gel-60 plates (Merck, Darmstadt, Germany) in heptane-isopropanol ether-glacial acetic acid (60:40:4, vol/vol/vol) with authentic standards. The bands corresponding to TG, FFA, DAG, and PL standards were scraped off the plate and transferred to screw cap glass tubes containing methylnpentadecanoic acid as an internal standard. FAs were then transmethylated in the presence of 14% boron trifluoride in methanol. The resulting methyl esters were extracted with hexane and analyzed by gas-liquid chromatography. Total lipid content was calculated from the individual FA content in each fraction.

Western blot analysis. The LV samples were homogenized and centrifuged at 10,500 g for 20 min in ice-cold 50 mM HEPES buffer.
LIPOGENESIS IN LEFT VENTRICULAR HYPERTROPHY

Table 1. Plasma parameters in Ex, Con, AAB, and sham rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Con</th>
<th>Ex</th>
<th>Sham</th>
<th>AAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>92.53 ± 9.09</td>
<td>89.31 ± 6.90</td>
<td>95.33 ± 5.54</td>
<td>99.81 ± 8.17</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>2.65 ± 0.18</td>
<td>1.91 ± 0.11*</td>
<td>2.78 ± 0.26</td>
<td>3.18 ± 0.41</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>56.17 ± 8.03</td>
<td>37.07 ± 5.59*</td>
<td>58.12 ± 6.68</td>
<td>60.25 ± 6.05</td>
</tr>
<tr>
<td>FFA, mM</td>
<td>0.35 ± 0.03</td>
<td>0.28 ± 0.02*</td>
<td>0.38 ± 0.03</td>
<td>0.36 ± 0.05</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>52.62 ± 4.21</td>
<td>40.52 ± 5.04*</td>
<td>49.33 ± 5.05</td>
<td>51.41 ± 3.91</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 6. Ex, endurance trained; Con, sedentary; AAB, abdominal aortic banded; FFA, free fatty acid; TG, triglyceride. *P < 0.05, Ex vs. Con.

(pH 7.4) containing 150 mM NaCl, 10 mM sodium pyrophosphate, 2 mM Na2VO4, 10 mM NaF, 2 mM EDTA, 2 mM PMSE, 5 μg/ml leupeptin, 1% Nonidet P-40, and 10% glycerol. Protein levels of SCD1, PPARα, CD36, FATP1, CPT1, AMPKα1 and -α2, DAGLα, DAGLβ, HSL, G0S2, ATGL, CGI-58, Akt, phosphorylated Akt, phosphorylated AMPK, phosphorylated HSL, and phosphorylated ACC were determined in 50 μg of clarified homogenate using specific antibodies. The separated proteins (9% SDS-PAGE gels) were transferred to PVDF membranes (Millipore) that were blotted using appropriate antibodies. The level of SREBP-1 protein was measured according to Park et al. (47). To measure the protein level of ACC, membranes were incubated for 1 h in streptavidin-horseradish peroxidase (Pierce) (38). The proteins were visualized using ECL (Pierce) as described by the manufacturer and quantified by densitometry.

Protein concentration. The protein concentration was determined using the Bio-Rad protein assay (Hercules, CA), using BSA as a standard.

Statistical analysis. The results were analyzed using Student’s t-test. A difference of P < 0.05 was considered significant. Values are presented as means ± SD.

RESULTS

Plasma parameters. The concentration of glucose in the plasma was not different between the sedentary and trained rats (Table 1). Compared with the control group, endurance training significantly decreased the plasma insulin (by 28%), cholesterol (by 34%), FFA (by 20%), and TG (by 23%) levels (Table 1). The concentrations of glucose, insulin, cholesterol, FFA, and TG in blood plasma were not different between the sham and AAB groups (Table 1).

Cardiovascular measurements. Six weeks of endurance training significantly increased the LV anterior and posterior wall thickness, whereas systolic and diastolic diameters as well as end-diastolic and -systolic volumes were significantly decreased (P < 0.05) in the trained group when compared with sedentary rats (Table 2). Also, the ejection fraction and fractional shortening were significantly increased in the heart with training-induced hypertrophy (Table 2). At 9 wk, abdominal aortic banding significantly increased LV anterior and posterior wall thickness by 18 and 53%, respectively, compared with the sham group. There was a significant LV systolic dysfunction in the AAB group, as seen in the increase in end-diastolic and -systolic volumes and reductions in the ejection fraction and fractional shortening when compared with the sham group (Table 2).

Expression of lipogenic genes and proteins. We assessed the mRNA and protein levels of factors that contribute to increased cellular assimilation of lipids. The mRNA levels of SREBP-1c, SCD1, SCD2, GPAT, and ACS and the protein levels of SREBP-1 and SCD1 were unchanged in the AAB group compared with the sham group, whereas these levels were increased (P < 0.05) in trained rats compared with sedentary controls (Fig. 1). These data show elevated expression of lipogenic genes in the myocardium of trained rats. SREBP-1 protein level and activity are regulated by Akt signaling (36). Therefore, we measured the level of phosphorylation of Akt in myocardium of the experimental animals. The ratio of phosphorylated Akt to Akt was twofold higher in trained rats compared with sedentary group, whereas AAB decreased Akt phosphorylation significantly (Fig. 1D). Obtained results suggest that Akt signaling is upregulated in physiological LV hypertrophy and is inactivated in AAB-induced hypertrophy.

AMPK pathway. Cardiac FA oxidation has been shown to be modulated by the activity of AMPK, which is activated by phosphorylation at Thr172 of the α-subunits (27). Therefore, we measured AMPK phosphorylation and AMPKα subunit pro-

Table 2. Echocardiographic analysis of heart function and structure of Ex, Con, AAB, and sham rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Con</th>
<th>Ex</th>
<th>Sham</th>
<th>AAB</th>
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<tbody>
<tr>
<td>HW/BW, g/g</td>
<td>0.20 ± 0.01</td>
<td>0.22 ± 0.01*</td>
<td>0.22 ± 0.01</td>
<td>0.28 ± 0.02*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>357.00 ± 14.54</td>
<td>330.00 ± 9.49</td>
<td>371.20 ± 22.72</td>
<td>354.20 ± 23.18#</td>
</tr>
<tr>
<td>AWT, mm</td>
<td>1.52 ± 0.04</td>
<td>1.68 ± 0.04*</td>
<td>1.82 ± 0.15</td>
<td>2.16 ± 0.23#</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>1.64 ± 0.05</td>
<td>1.82 ± 0.08*</td>
<td>1.86 ± 0.17</td>
<td>2.86 ± 0.11#</td>
</tr>
<tr>
<td>EDD, mm</td>
<td>7.50 ± 0.20</td>
<td>7.26 ± 0.37*</td>
<td>7.82 ± 0.26</td>
<td>9.22 ± 0.54#</td>
</tr>
<tr>
<td>ESD, mm</td>
<td>4.06 ± 0.19</td>
<td>3.76 ± 0.28*</td>
<td>4.22 ± 0.28</td>
<td>5.78 ± 0.32#</td>
</tr>
<tr>
<td>RWT</td>
<td>0.44 ± 0.02</td>
<td>0.50 ± 0.03*</td>
<td>0.48 ± 0.05</td>
<td>0.62 ± 0.03#</td>
</tr>
<tr>
<td>EDV, ml</td>
<td>0.44 ± 0.04</td>
<td>0.40 ± 0.06*</td>
<td>0.50 ± 0.05</td>
<td>0.83 ± 0.14#</td>
</tr>
<tr>
<td>ESV, ml</td>
<td>0.07 ± 0.01</td>
<td>0.06 ± 0.01*</td>
<td>0.08 ± 0.02</td>
<td>0.20 ± 0.03#</td>
</tr>
<tr>
<td>EF, %</td>
<td>84.07 ± 1.99</td>
<td>86.03 ± 2.17*</td>
<td>84.14 ± 2.82</td>
<td>75.01 ± 4.62#</td>
</tr>
<tr>
<td>FS, %</td>
<td>0.46 ± 0.02</td>
<td>0.48 ± 0.03*</td>
<td>0.46 ± 0.03</td>
<td>0.37 ± 0.04#</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 6. Ex, endurance trained; HW/BW, heart weight/body weight × 100; HR, heart rate; AWT, anterior wall thickness; PWT, posterior wall thickness; EDD, end-diastolic diameter; ESD, end-systolic diameter; RWT, relative wall thickness; EDV, end-diastolic volume; ESV, end-systolic volume; EF, ejection fraction [EF = (EDV − ESV)/EDV]; FS, fractional shortening [FS = (LVED − LVSD)/LVED, where LVED is left ventricular diameter in diastole and LVSD is left ventricular diameter in systole]. *P < 0.05, Ex vs. Con; #P < 0.05, AAB vs. sham.
tein levels in heart homogenates. AMPK protein levels were not affected by either exercise training or AAB (Fig. 2A). AMPK phosphorylation of the α-subunit was increased in both trained and AAB groups compared with appropriate controls (Fig. 2A). Phosphorylation of ACC at Ser79 by AMPK leads to the inhibition of ACC activity (27, 38). Consistent with the increased AMPK phosphorylation that was observed after training and AAB, ACC phosphorylation was also increased significantly in the hearts of exercised and AAB rats (Fig. 2B), additionally confirming the activation of the AMPK pathway. The ACC protein level was not affected by training or AAB (Fig. 2B).

The rate of FA β-oxidation is controlled by their rate of transfer into the mitochondria through CPT I (4, 55). To address this process, we measured CPT I mRNA and protein levels in the hearts of trained, sedentary, AAB, and sham rats.

Fig. 1. Expression of the lipogenic genes (A), protein levels of sterol regulatory element-binding protein-1c (SREBP-1c; B) and stearoyl-CoA desaturase 1 (SCD1; C), and Akt protein and phosphorylation levels (D) in the heart of endurance-trained (Ex), sedentary (Con), abdominal aortic-banded (AAB), and sham rats. SREBP-1c, SCD1 and -2, glycerol-3-phosphate transferase (GPAT), and acyl-CoA synthase 1 (ACS) mRNA levels were measured by real-time PCR. Protein levels of SREBP-1c, SCD1, and Akt were determined by Western blot analysis. *P < 0.05, Ex vs. Con; #P < 0.05, AAB vs. Sham; n = 6.

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CPT I protein and mRNA levels were increased two- and 1.3-fold, respectively, in the myocardium of trained rats relative to untrained rats (Fig. 2C and 3A). The level of CPT I protein was also increased, but to a lesser extent, in the hearts of AAB rats compared with sham rats (Fig. 2C), although the CPT I mRNA level was not affected by AAB (Fig. 3A).

**PPARα pathway.** Another important factor responsible for transcriptionally regulating genes of FA catabolism, including CPT I, is PPARα, a transcription factor expressed predominantly in liver and heart (20, 30). When activated, this transcription factor promotes the expression of FA oxidation genes (20). Because we observed an increased level of CPT I mRNA in trained animals, we were interested in determining whether these changes were mediated by increased PPARα activity. Therefore, we measured the expression of two genes that are regulated by PPARα, ACO and PPARα itself. As expected, the mRNA levels of ACO were increased significantly in the hearts of trained rats (Fig. 3A). The mRNA and protein levels of PPARα were also increased 1.5- and 2.9-fold, respectively, in the hearts of trained rats compared with untrained rats (Fig. 3, A and B), suggesting increased PPARα activity. Interestingly, neither PPARα nor ACO expression was elevated in the myocardium of AAB rats (Fig. 3, A and B).

To shed light on the molecular mechanism of PPARα upregulation in the heart of exercised rats, we analyzed the level of polyunsaturated fatty acids (PUFAs), which are very potent endogenous regulators of PPARα activity (56). The intracellular content of PUFAs was increased by 32% in the heart of trained compared with sedentary rats (Fig. 3C). Thus...
it is possible that the activation of PPARα in trained hearts is associated with increased intracellular PUFAs. Consistently, PUFAs content was not changed in the hearts of AAB compared with sham rats (Fig. 3C).

Cardiac lipid content. To assess the effects of the changes in the expression of lipogenic and oxidative proteins on the cardiac lipid content, we measured the levels of FFAs, DAG, TG, and PLs in the hearts of trained, sedentary, AAB, and sham rats. Exercise training did not affect cardiac FFA, DAG, TG, or PL concentrations (Fig. 4). AAB increased cardiac TG (by 31%) and DAG (2-fold) levels significantly, whereas AAB did not affect FFA or PL concentrations in the heart (Fig. 4).

FATPs. FA uptake by cardiac myocytes occurs by two main transport processes: protein-mediated transport, which accounts for ~80% of the total FA uptake, and simple diffusion (23). CD36 and FATP are the major proteins responsible for membrane FA transport in the heart (10). Cardiac-specific overexpression of FATP1 was shown to increase lipid accumulation in the heart and to cause lipotoxic cardiomyopathy (9). To elucidate the potent mechanism that leads to TG and DAG accumulation in the AAB hearts, we measured CD36 and FATP1 protein levels by Western blot analysis and FATP mRNA levels by real-time PCR. The mRNA and protein levels of FATP were induced by endurance training (Fig. 5, B and C). AAB did not affect FATP expression, whereas AAB decreased the CD36 protein level (Fig. 5). Thus, elevated membrane FA transport is likely not responsible for the TG and DAG accumulation in the hearts of the AAB group.

Lipolytic enzymes. ATGL catalyzes the hydrolysis of TG, the rate-limiting step in lipolysis in the heart (57). G0S2 inhibits TG hydrolase activity of ATGL, whereas CGI-58 activates the enzyme (74). ATGL protein levels were not different between trained and sedentary rats as well as between AAB and sham rats (Fig. 6A). Interestingly, the protein level of ATGL inhibitor G0S2 was increased in AAB group compared with sham. ATGL activator CGI-58 protein level was ~80% higher in trained rats than in control rats (Fig. 6A). These results underline the important differential role of ATGL in cardiac lipid metabolism and suggest a different mechanism regulating ATGL activity in pathological and physiological hypertrophy.

ATGL generates DAG, which is subsequently hydrolyzed by HSL (57). Protein kinase A phosphorylates HSL at Ser563, which stimulates HSL activity (1, 28). In contrast, AMPK phosphorylates HSL at Ser565, which inhibits HSL activity (1, 22). The level of HSL protein was not affected by either exercise or AAB relative to appropriate controls (Fig. 6B). Endurance training decreased the phosphorylation of HSL at Ser563, indicating the activation of the enzyme and increased lipolysis. Conversely, AAB increased the phosphorylation of HSL at Ser565 (Fig. 6B), suggesting reduced enzymatic activity of HSL. In both cases, the phosphorylation of HSL at Ser563 remained unchanged.

DAGL converts DAG to monoacylglycerol and a fatty acid and thus, besides HSL, can attenuate DAG levels (31). Therefore, we measured DAGLα and DAGLβ protein levels in the heart of trained, untrained, AAB, and sham rats. DAGL protein levels were unchanged in trained rats compared with sedentary controls (Fig. 6C). However, DAGLα and DAGLβ protein levels were decreased by 18 and 26%, respectively (Fig. 6C), in the myocardium of AAB rats relative to sham rats.

Taken together, all these data suggest that TG and DAG hydrolysis are increased in training-induced hypertrophy and reduced significantly in the heart with AAB-induced hypertrophy.

DISCUSSION

Intracellular lipid accumulation and the rate of fatty acid utilization in the heart are closely related to cardiac function...
was measured by real-time PCR. *P of Ex, Con, AAB, and sham rats. Protein levels of FAT/CD36 and FATP1 confirmed the role of this pathway in heart hypertrophy (11, 39). Although the physiological and morphological changes during cardiac adaptations to hypertrophy are well characterized (52, 63, 69, 70), little is known about the molecular changes in lipid biosynthesis and accumulation. In the present study, for the first time, we show that the expression of lipogenic genes is increased in exercise-induced but not in pressure overload-induced hypertrophy and suggest that activation of lipogenesis may play a role in the mechanism underlying the differences between physiological and pathological cardiac hypertrophy.

The mRNA expression and protein level of SREBP-1c, an insulin-regulated lipogenic transcription factor, and its target genes SCD1, SCD2, GPAT, and ACS were increased significantly in the LV of the heart with training-induced hypertrophy. None of the analyzed lipogenic genes nor SREBP-1c was upregulated in the heart with pathological hypertrophy induced by AAB. Accumulation of mature SREBP-1 and expression of SREBP-1c target genes may be regulated by Akt (48). The link between Akt signaling and cardiac hypertrophy was established first by observation of Akt activation in cultured cardiomyocytes (43). Subsequent studies on heart-specific murine models of either overexpressed or knocked down Akt further confirmed the role of this pathway in heart hypertrophy (11, 41). We found Akt pathway to be upregulated in training-induced hypertrophy and reduced in AAB-induced hypertrophy. These results are consistent with studies that have suggested that activation or inactivation of cardiac Akt signaling diverges physiological from pathological LV hypertrophy (32). Considering that Akt can modulate the activity of SREBP-1c (37, 48) upregulated Akt signaling may be poised for increased lipogenesis in physiological hypertrophy.

Surprisingly, activation of the SREBP-1c pathway was not associated with lipid accumulation in the trained heart. Various SREBP-1 transgenic mice develop fatty liver, β-cell failure, and other lipotoxic phenotypes (62). However, the myocardium has a marginal capacity for de novo lipogenesis, suggesting that increased cardiac SREBP-1, SCD1, FAS, and ACS in the trained heart might elicit responses distinct from other tissues. Lipogenesis consumes energy to synthesize fat; thus, activation of this process in the settings of increased myocardial energy demand, as in the case of exercise training, must be worth its energy cost; e.g., the SREBP-1c pathway might be involved in the regulatory mechanisms of heart adaptation to stress stimuli. In support of this notion, cardiac SREBP-1 was shown to activate the G protein-coupled inwardly reflecting K+ channel (GIRK1/4), leading to enhanced acetylcholine-sensitive K+ currents and reduced arrhythmias post-myocardial infarction (46). Additionally, myocardial FAS and SCD1 were shown to be involved in the regulation of the ability of the heart to respond to stress through a process that appears to involve the activation of Ca2+/calmodulin-dependent protein kinase II (50) and cause a shift in substrate metabolism toward a preference for glucose utilization (14, 16), respectively. Thus it is possible that increased lipogenesis is the first and necessary step in the development of maladaptive LV hypertrophy, and a lack of activation of lipogenic genes, as we found in AAB-induced LV hypertrophy, may lead to pathological events that eventually result in heart dysfunction.

Potential roles for endogenous lipids, such as those produced by FAS, SCD, or GPAT, in the regulation of transcription factor activity and the rate of cardiac energy substrate utilization were reported previously by us (13) and others (18, 50). Here, we found increased mRNA expression and protein levels of SCD1 in LV with training-induced hypertrophy. Oleate that is endogenously synthesized by SCD was shown to activate cardiac FA β-oxidation by increasing AMPK activity (13). Consistently, in training-induced hypertrophy, the phosphorylation of AMPK was increased significantly. Elevated AMPK phosphorylation was associated with a physiological AMPK effect, i.e., increased ACC phosphorylation. This increased phosphorylation, together with increased CPT I protein and mRNA levels, might lead to an elevated rate of FA β-oxidation because ACC is an important regulator of malonyl-CoA concentrations that modulate CPT I activity and in turn the rate of FA oxidation in both heart (38) and skeletal muscle (53). Simultaneously, we found that endurance training increases the mRNA and protein levels of FATP1, potentially contributing to increased FA uptake, and enhances the activity of PPARα, a major mediator of myocardial lipid metabolism (21). The upregulation of PPARα in the trained group was associated with a 32% increase in the amount of PUFAs, which are very potent endogenous regulators of PPARα activity (56). Increased PPARα, CPT I, and ACO expression in the heart may contribute to the improvement of the FA metabolic enzyme.
activities in trained hearts and play a role in the adaptive mechanism of exercise-induced hypertrophy (30, 52, 70). Conversely, in some of the experimental models of cardiac failure, the expression of PPARγ was shown to be diminished (2, 44, 51, 54). However, no changes in PUFA content or in PPARγ protein and mRNA levels were observed in the heart with AAB-induced hypertrophy. In fact, various studies reported marginal changes, if any, in FA oxidation rates in ex vivo perfused hypertrophied hearts (51, 58, 75) and unaltered oxygen consumption and substrate uptake rates in the hypertrophied myocardium in situ (59). Moreover, in the early stages of heart failure in the human heart, there is a normal (or slightly elevated) rate of FA utilization, with a dramatic downregulation of FA oxidation in advanced or end-stage heart failure (34).

Hypertrophy induced by AAB was accompanied by increased levels of both TG and DAG in the myocardium in our study. Noteworthy is that no differences in circulating FFA, TG, or cholesterol were observed between AAB and sham groups. Because hearts with AAB-induced hypertrophy had neither detectable changes in the expression of lipogenic genes nor an increased level of FA transporters (CD36, FATP1), it is unlikely that TG and DAG accumulation was prompted by a gross increase in de novo lipogenesis or cellular FA uptake. Many studies show that TGs are a major source of FAs for the murine heart (3, 39, 65) and that TG hydrolysis plays a central role in mediating cardiac metabolism and function in both the healthy and diseased heart (25, 26, 33, 40, 49, 60). ATGL and HSL are rate-limiting enzymes in TG and DAG hydrolysis in the heart (57). Mice with global targeted deletion of ATGL develop severe lipotrophic cardiomyopathy (25). Similarly, mutations in the gene coding ATGL (PNPLA2) in humans lead to myocardial steatosis, cardiomyopathy, and heart failure (60). Thus increased levels of the ATGL inhibitor G0S2 may result in reduced TG hydrolysis and, in turn, increased lipid accumulation in AAB-induced hypertrophy. HSL activity is regulated by AMPK (1, 22). Activation of AMPK has been observed in
pathological hypertrophy, but its role is not completely understood (66, 76). Studies performed on cultured cardiac myocytes indicated that the activation of AMPK attenuates hypertrophy (7). In our study, phosphorylation of AMPK and its target proteins, i.e., ACC(Ser79) and HSL(Ser655), was increased significantly in the heart with AAB-induced hypertrophy. Interestingly, despite increased phosphorylated AMPK, the level of p-HSL(Ser655) was reduced in the trained group probably because of increased activity of phosphatase 2A (36). Phosphorylation of HSP at Ser655 by AMPK inhibits the enzyme and leads to reduced hydrolysis of TG and DAG (1, 22). Moreover, the levels of myocardial DAGLα and DAGLβ, which are major lipases involved in DAG hydrolysis (31), were reduced in this model of hypertrophy. Taken together, all of these data suggest that the hydrolysis of TG and DAG is reduced significantly in AAB-induced hypertrophy. Because reduction in cardiac lipid hydrolysis is accompanied by intracellular TG and DAG accumulation (25, 60), it is likely that increased TG and DAG levels in AAB-induced hypertrophy are a result of decreased lipolysis.

Cardiac lipid deposition may have negative effects in the heart, leading to cardiomyopathy and heart failure. This lipotoxic impact of lipids was initially shown in various animal models of obesity (67) but more recently has also been shown in lean rodent models with targeted overexpression of genes involved in lipid delivery and synthesis in the myocardium (8, 9, 42, 73). In humans, increased amounts of lipid deposits were found in the myocardium of patients with left ventricular hypertrophy and the metabolic syndrome (40). DAG might play an important role in cardiac lipotoxicity because DAG-induced activation of protein kinase C in skeletal muscle, aorta, and cardiac cells is associated with insulin resistance and was proposed as a factor leading to heart failure (24, 45, 72). A recent study showed that cardiacmyocyte-specific overexpression of TG hydrolyase improved systolic function and protected the heart from pressure overload-induced cardiac dysfunction (33) and diabetes-induced lipotoxic cardiomyopathy (49). In contrast, downregulation of ATGL severely disrupted mitochondrial substrate oxidation and respiration in the heart; this was followed by excessive lipid accumulation, cardiac insufficiency, and lethal cardiomyopathy (26). It is thus possible that reduced lipolysis leading to increased TG and DAG levels in the early stages of AAB-induced LV hypertrophy may hamper cardiac function and predispose to cardiomyopathy and heart failure in the future.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
P.D. and A.D. contributed to the conception and design of the research; P.D., A.P., M.K.D., T.B., M.M., and J.L. performed the experiments; P.D. analyzed the data; P.D. interpreted the results of the experiments; P.D. prepared the figures; P.D. drafted the manuscript; P.D. and A.D. edited and revised the manuscript; P.D., A.P., M.K.D., T.B., M.M., J.L., and A.D. approved the final version of the manuscript.

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