Decreased rates of substrate oxidation ex vivo predict the onset of heart failure and contractile dysfunction in rats with pressure overload

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Aims
Left ventricular hypertrophy is a risk factor for heart failure. However, it also is a compensatory response to pressure overload, accommodating for increased workload. We tested whether the changes in energy substrate metabolism may be predictive for the development of contractile dysfunction.

Methods and results
Chronic pressure overload was induced in Sprague–Dawley rats by aortic arch constriction for 2, 6, 10, or 20 weeks. Contractile function in vivo was assessed by echocardiography and by invasive pressure measurement. Glucose and fatty acid oxidation as well as contractile function ex vivo were assessed in the isolated working heart, and respiratory capacity was measured in isolated cardiac mitochondria. Pressure overload caused progressive hypertrophy with normal ejection fraction (EF) at 2, 6, and 10 weeks, and hypertrophy with dilation and impaired EF at 20 weeks. The lung-to-body weight ratio, as marker for pulmonary congestion, was normal at 2 weeks (indicative of compensated hypertrophy) but significantly increased already after 6 and up to 20 weeks, suggesting the presence of heart failure with normal EF at 6 and 10 weeks and impaired EF at 20 weeks. Invasive pressure measurements showed evidence for contractile dysfunction already after 6 weeks and ex vivo cardiac power was reduced even at 2 weeks. Importantly, there was impairment in fatty acid oxidation beginning at 2 weeks, which was associated with a progressive decrease in glucose oxidation. In contrast, respiratory capacity of isolated mitochondria was normal until 10 weeks and decreased only in hearts with impaired EF.

Conclusion
Pressure overload-induced impairment in fatty acid oxidation precedes the onset of congestive heart failure but mitochondrial respiratory capacity is maintained until the EF decreases in vivo. These temporal relations suggest a tight link between impaired substrate oxidation capacity in the development of heart failure and contractile dysfunction and may imply therapeutic and prognostic value.

Keywords
Echocardiography • Heart failure • Substrate oxidation predictive for diagnosis of heart failure • Pressure overload

1. Introduction
Chronic pressure overload leads to myocardial hypertrophy. Although this increase in left ventricular mass may be an effective compensatory mechanism to generate the required cardiac output under conditions of increased workload for years, the presence of hypertrophy itself is a risk factor for the development of heart failure.1 In clinically relevant scenarios of chronic pressure overload (e.g. aortic stenosis), increased left ventricular mass precedes heart failure and may therefore be maladaptive rather than beneficial.2 Several mechanisms for hypertrophy and heart failure have already been identified, including abnormal signal transduction,3 mitochondrial dysfunction,4 and disrupted intracellular calcium handling.5 Most of these mechanisms affect energy substrate utilization, which is required to generate ATP for contraction. In heart failure, normal6 or decreased fatty acid oxidation rates,7 as well as shifts towards glucose oxidation have been described. However, no study has truly quantified glucose and fatty acid utilization during the development of heart failure.

Neubauer8 recently proposed an intriguing metabolic concept for heart failure that still awaits full experimental proof. In the early
phase of heart failure, a shift from predominant fatty acid oxidation towards predominant glucose oxidation (presumably a compensatory mechanism to support a more efficient ATP production) is followed by a decline in overall substrate oxidation. Based on this hypothesis, it should be possible to determine the stage of hypertrophy/heart failure by the heart’s substrate oxidation pattern.

We therefore created chronic pressure overload-inducing hypertrophy and heart failure, measured contractile function, and hypertrophy grade in vivo and ex vivo, quantified substrate oxidation rates in the isolated working heart, and measured respiratory capacity of isolated mitochondria. We found that the abnormal substrate oxidation pattern may predict the development of heart failure. To study this predictive ability of substrate oxidation, it is crucial to define heart failure including its onset.

2. Methods

2.1 Animals
Male Sprague–Dawley rats were obtained from Charles River (Sulzfeld, Germany) and were fed ad libitum at 21°C with a light cycle of 12 h. The animals were 3 weeks of age (40–50 g). The use of animals was consistent with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996) and the experimental protocols were approved by the local Animal Welfare Committee of the University of Freiburg and Leipzig, Germany (AZ: 24-9168.11TVV/36/06).

2.2 Materials
Chemicals were obtained from Sigma Aldrich (Deisenhofen), Merck (Darmstadt), Serva (Heidelberg), Essex (München), Bayer (Leverkusen), Narkodorn (Neumünster), and BIO-RAD (München).

2.3 Study design
Animals were subjected to chronic pressure overload at 3 weeks of age (see Section 2.4) and were followed for the next 20 weeks. We did not perform additional investigations at time points beyond 20 weeks, because animals were in end-stage heart failure at 20 weeks and the fraction of animals surviving an additional 1 or 2 weeks would be minimal. At 2, 6, 10, and 20 weeks, animals were used for further analysis. Due to the nature of the analyses, it was not possible to perform all experiments in all animals. Therefore, subsets were assigned to the individual methodology described below. The final group sizes for the specific analyses are given in detail in the Figures and Tables. These analyses included echocardiographic determination of contractile function and heart size in vivo, assessment of substrate oxidation rates and contractile function ex vivo in the isolated working heart system, measurement of mitochondrial respiratory capacity, and determination of heart failure markers by RT-PCR as described in detail below.

2.4 Surgical interventions
The model of hypertrophy and heart failure has been described in detail earlier. Pressure overload was created by placement of a metal clip around the aortic arch between the brachiocephalic trunk and the left carotid artery at a weight of 40–50 g. The clip had a remaining opening of 0.35 mm and was applied to aortic arch in the anesthetized and intubated rat through a partial sternotomy. Age-matched sham-operated rats were extubated and kept on warming blankets until full consciousness was regained.

2.5 Clinical assessment and echocardiography
Rats were weighed and inspected weekly. Echocardiographic examination was performed after 2, 6, 10, and 20 weeks postoperatively. The animals were anesthetized with Fentanyl/Midazolamhydrochlorid/Medetomidinhydrochlorid (0.005/2/0.15 mg/Kg). Chests were shaved and the rats were examined in supine position with a 12-MHz phased array transducer (Aglilent/Philips, Germany). Two-dimensional short-axis views of the left ventricle at papillary muscle level were obtained. Two-dimensional guided M-mode tracings were recorded with a sweep speed of 100 mm/s. The following parameters were measured: heart rate (HR), interventricular septal thickness in diastole (IVSd), left ventricular end diastolic dimension (LVEDD), left ventricular posterior wall thickness in diastole (LVPWd), interventricular septal thickness in systole (IVSs), left ventricular end systolic dimension (LVESD), left ventricular posterior wall thickness in systole (LVPWs), early transmural wave (E wave), and early transmural wave/early diastolic wave at basal interventricular septum by pulse-wave tissue doppler (E/E’). Based on these measurements, the following parameters were determined: ejection fraction (EF), left ventricular endocardial fractional shortening (FS), calculated LV mass (LVM), LV mass index (LVMi), posterior wall thickening (PWT), relative wall thickness (RWT), inner shell–inner 1/2 shell of myocardium, end-diastolic volume (EDV-LV), end-systolic volume (ESV - LV).

2.6 Invasive assessment of aortic pressures
Invasive pressure measurement was performed as described before. Shortly, after anaesthesia, the femoral and the carotid arteries were dissected and a 1.4F Millar catheter was inserted and advanced into the abdominal aorta or the left ventricle and data were recorded. After pressure recordings were completed hearts were excised for further analysis.

2.7 Organ harvesting and determination of the lung-to-body weight index
At the end of the observation periods and after echocardiography, animals were weighed, weighted, and prepared for perfusion as isolated hearts. Both lungs were excised at the hilus and weighed. The lung-to-body weight index (LBI) was calculated as lung wet weight (g) to body weight (kg). The presence of pleural effusions was also recorded during heart harvesting for isolated heart perfusions. Pleural effusions were considered significant, if there was a visible amount of fluid in the pleural cavities after opening that exceeded the physiological level.

2.8 Isolated working heart perfusion and assessment of substrate oxidation rates
The preparation is those developed by Taegtmeyer et al. and has been described by us in detail earlier. Briefly, rats were anesthetized with sodium pentobarbital (5 mg/100 g body wt i.p.). After injection of heparin (200 IU) into the inferior vena cava, the heart was rapidly removed and placed in ice-cold Krebs–Henseleit bicarbonate buffer. The aorta was freed of excess tissue and cannulated. A brief period of retrograde perfusion (less than 5 min) with oxygenated buffer containing glucose (10 mM) was necessary to wash out any blood from the heart and to perform left atrial cannulation. Hearts were then perfused as working hearts at 37°C with recirculating Krebs–Henseleit buffer (200 mL) containing 1% bovine serum albumin, Cohn fraction V, fatty acid free (Sigma Chemicals Co., St Louis, MO, USA). Perfuse calcium concentration was 2.5 mM. Hearts were perfused with glucose (5.0 mmol/L) and oleate (0.4 mmol/L) as substrates. The perfuse was gassed with 95% O2—5% CO2, and recirculated. All experiments were carried out with a preload of 15 cmH2O and an afterload of 100 cmH2O. The hearts were beating spontaneously at a rate of...
approximately 250 beats/min. After stabilization, hearts were perfused for a 30-min period, in which all samples were withdrawn and measurements performed. Aortic flow and coronary flow were measured every 5 min by timing the rise of the fluid meniscus in a calibrated glass tube.21 Cardiac output (mL/min) was calculated as the sum of aortic and coronary flow. Heart rate and pressure were measured continuously with a Hugo Sachs transducer and recording system (Hugo Sachs, March, Germany). Cardiac power was determined as the product of cardiac output and mean aortic pressure as described before.20 Glucose and fatty acid oxidation rates were determined from the production of $^{14}C_{2}$CO$_{2}$ from $[^{9,10-3H}]$oleate.21 At the end of perfusion, hearts were freeze-clamped for further analysis.

Cardiac power per ATP was calculated from cardiac power measured and from exogenous substrate oxidation rates, based on 31 mole ATP produced per mole glucose and 118.5 mole ATP per mole oleate oxidized.22

2.9 Isolation of mitochondria
Cardiac mitochondria are isolated according to Palmer et al.23 except that a modified Chappell–Perry buffer (containing 100 mM KCl, 50 mM MOPS, 1 mM EGTA, 5 mM MgSO$_{4}$$\cdot$7H$_{2}$O, and 1 mM ATP, pH 7.4, 4°C) was used. Mitochondria were harvested following treatment of the homogenate with 5 mg/g heart weight wet trypsin for 10 min at 4°C.24 Mitochondrial protein concentration was determined by the Bradford method using bovine serum albumin as a standard.

2.10 Mitochondrial oxidative phosphorylation
Oxygen consumption of isolated mitochondria was measured using a Clark-type oxygen electrode (Strathkelvin) at 25°C.25 Mitochondria were incubated in a solution consisting of 80 mM KCl, 50 mM MOPS, 1 mM EGTA, 5 mM K$_{2}$HPO$_{4}$, and 1 mg/mL fatty acid-free bovine serum albumin at pH 7.4. The rate of oxidative phosphorylation was measured using glutamate and substrate as ADP and stimul. As the ADP-stimulated oxygen consumption (state 3) and the ADP-limited oxygen consumption (state 4) in the respiratory chamber and the ADP/O ratio (ADP added per oxygen consumed) were determined as previously described.17 Forward and reverse primers were designed using the Universal Probe Library Assay design center. For each set of primers, a basic local alignment search tool (BLAST) search revealed that sequence homology was obtained only for the targeted gene. PCR amplification was performed in triplicates in a reaction volume of 10 µL using AmpliTaq Gold (Applied Biosystems, Darmstadt, Germany) with the conditions suggested by the manufacturer. After initial denaturation and activation of enzyme for 10 min at 95°C 40 cycles of denaturation at 95°C for 15 s, annealing and extension at 60°C for 1 min were run. Primers used were: ANF forward: TGGATGAAGCTGACGCTGG reverse: CGTCCCTATCTTTGCAACG probe: Universal Probe Library (UPL) #67; BNP forward: AGAACAATCCAGTGACGAGA reverse: GCTG TTCTGAGCCCATTTCCC probe: UPL #67; α-MHC forward: CAGAAAG AAACTGAAGGAAGACCA reverse: GCTCGGCTCTTCTCTCTCT probe: UPL #17; β-MHC forward: CAG CCT ACC TCA TGG GAC TGA reverse: GTG ACA TAC TCG TTG CCC ACT TT probe: 6-FAM-CTG TGC CAC CCT CGA GAC TGA G reverse: GTG ACA TAC TCG TTG CCC ACT TT probe: 6-FAM-CTG CAA GGG CCT TCT TCG CGG-CCG A A probe: FABP forward: TTT GTC GGT ACC TGG AAC GTA ACA CTA reverse: CAT CGT GTG GGT GAA G probe: UPL #56; FAT forward: TTA CGT GAG CGG TTA TTA GT G reverse: TCC TTC TTC AAG GAC AAC TTC C probe: UPL #76; LCAD forward: GCA GTT ACT TGG GAA GAG CAA reverse: GATG ACA ATA TCT GAA TGG A probe: UPL #81; MCD forward: GGAC ACT AGG GTG TTT CCT CCT GAG reverse: CGG AAC ATT TGT TTG AAA CTC probe: UPL #84; mCPT1 forward: AGT GTG CCA GCC ACA ATT CA reverse: ATA GCC TTC GTC ATC CAG CAA probe: 6-FAM-CCG TAC TCG GAT TCT GTG C-MGB. A standard series of five dilutions was used for quantification. Results were normalized to S29 transcription as a housekeeping gene product, which was not different among all samples.

2.12 Statistical analysis
Data are presented as mean ± SEM. Data were analysed using a one-way analysis of variance or a student t-test where appropriate. Post-hoc comparisons among the groups were performed using the Holm–Sidak method.28 Differences among groups were considered statistically significant if P < 0.05.

| Table 1 Heart, body, and atrial weights as well as tibia length of animals after 2, 6, 10, and 20 weeks of pressure overload compared with control |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                | 2 Weeks        | 6 Weeks        | 10 Weeks       | 20 Weeks       |
|                | (n = 14)       | (n = 14)       | (n = 12)       | (n = 20)       | (n = 11)       | (n = 10)       |
| BW (g)         | 145 ± 5        | 119 ± 7**     | 272 ± 7        | 258 ± 7        | 348 ± 7        | 342 ± 10       | 387 ± 15.5     | 420 ± 7        |
| HW wet (g)     | 0.85 ± 0.03    | 0.88 ± 0.07   | 1.50 ± 0.05    | 2.20 ± 0.05**  | 1.52 ± 0.22    | 2.65 ± 0.07*** | 1.85 ± 0.07    | 3.74 ± 0.09*** |
| HW dry (g)     | 0.12 ± 0.00    | 0.14 ± 0.01*  | 0.23 ± 0.08    | 0.35 ± 0.02*** | 0.23 ± 0.03    | 0.44 ± 0.01*** | 0.28 ± 0.01    | 0.64 ± 0.03*** |
| HW dry/BW (mg/g) | 0.85 ± 0.02   | 1.22 ± 0.05*** | 0.85 ± 0.04    | 1.37 ± 0.02*** | 0.72 ± 0.02    | 1.29 ± 0.04*** | 0.70 ± 0.02    | 1.53 ± 0.07*** |
| Tibia length (mm) | 27.3 ± 0.4  | 23.9 ± 0.5*** | 33.2 ± 0.6     | 34.3 ± 0.3     | 37.7 ± 0.4     | 38.0 ± 0.2     | 39.4 ± 1.0     | 41.3 ± 0.8     |
| HW dry/TL (mg/mm) | 4.3 ± 0.1  | 6.0 ± 0.2***  | 6.9 ± 0.3      | 10.3 ± 0.3***  | 6.5 ± 0.2      | 11.2 ± 0.3***  | 7.1 ± 0.4      | 15.6 ± 0.6***  |
| Atrial weight (mg) | 77 ± 7    | 76 ± 7        | 120 ± 22       | 294 ± 19***    | 109 ± 10       | 326 ± 33***    | 156 ± 25       | 537 ± 58***    |

Data are mean ± SEM. BW, body weight; HW, heart weight; TL, tibia length; PO, pressure overload.

*a P < 0.05, **P < 0.01, ***P < 0.001, compared with age-matched controls after 2, 6, 10, 20 weeks of pressure overload by aortic constriction.
3. Results

The banding procedure was well tolerated (operative mortality 5%). Animals demonstrated normal growth after a 3–4-day recovery period. Clinical signs of heart failure (dyspnea, pleural effusions, blue extremities, and inactivity) became apparent between 19 and 20 weeks of pressure overload.

3.1 Pressure overload causes compensated hypertrophy followed by heart failure with normal and then impaired EF

Table 1 shows basic morphometric parameters of animals analysed 2, 6, 10, and 20 weeks after banding. Heart weights as well as heart weight to body weight ratios in all groups were higher in the banded groups than in the age-matched controls, indicating a significant degree of hypertrophy induced by the banding procedure in all groups. The same was true for relating heart weight to tibia length. Atrial weight was normal after 2 weeks and increased progressively thereafter, indicating progressive congestion.

Figure 1 shows the key echocardiographic measurements of the hearts at the different time points compared with their age-matched controls. Table 2 lists the remaining echocardiographically assessed parameters. Posterior wall thickness (Panel A) and left ventricular mass index (Table 2) were significantly elevated 2, 6, and 10 weeks after banding, indicative of hypertrophy. Posterior wall thickness had normalized 20 weeks after banding but LVM remained high and left ventricular end diastolic diameter (Panel B) had increased, indicative of left ventricular dilation. The change in LVEDD was inversely related to the EF (C) and FS (D), which were normal 2, 6, and 10 weeks after banding, but significantly decreased after 20 weeks. The dilation of the ventricle and the impairment of EF correlated well with the onset of clinical heart failure symptoms, as evident by dyspnea, rough fur, and inactivity. We also assessed diastolic function measuring the E wave and E/E' in our animals. Both parameters were normal up to 6 weeks and significantly reduced after 10 and 20 weeks.

Table 3 shows the results of invasive Millar catheter measurements. There were significant pressure gradients over the stenosis caused by the constriction at all time points. However, the gradient varied substantially over time, being highest at 20 weeks and lowest at 10 weeks without a clear pattern of change or progression. Due to the influence of systolic developed pressure on the assessment of the pressure change over time, we normalized dp/dt to LVDP as suggested by Schmidt and Scheer.29 These results demonstrate a normal indexed

![Figure 1](https://example.com/figure1.png)

**Figure 1** Left ventricular posterior wall thickness in diastole (LVPWD, A), left ventricular end diastolic dimension (LVEDD, B), ejection fraction (EF, C), and left ventricular fractional shortening (FS, D) of rat hearts subjected to 2, 6, 10, or 20 weeks of pressure overload compared with age-matched control. Data are mean ± SEM; *P < 0.05, **P < 0.01; numbers in the columns indicate group size (n).
dp/dt at 2 weeks and impairment already after 6 weeks, which remained low up to 10 and 20 weeks. We also calculated wall stress (data not shown) which was normal at 2 weeks, mildly elevated at 6 and 10 weeks, and doubled at 20 weeks.

Figure 2 shows the lung-to-body weight ratios as marker for pulmonary congestion. Supportive of the invasive pressure measurements, lung-to-body weight ratios were already elevated at 6 weeks and remained high thereafter. Beginning at 6 weeks animals displayed additional significant pleural effusions in 15% of the cases, which increased to 33% at 10 and 75% at 20 weeks of pressure overload, indicative of progressive worsening of heart failure.

The above results suggest that our model of pressure overload in rats caused a state of compensated hypertrophy (up to 2 weeks) followed by heart failure with normal EF (6 and 10 weeks) and heart failure with impaired EF (20 weeks). This terminology will be used for the remainder of the manuscript.

Table 2 Echocardiographic parameters of hearts subjected to pressure overload

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<th>2 Weeks</th>
<th>6 Weeks</th>
<th>10 Weeks</th>
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<tr>
<td></td>
<td>Control</td>
<td>PO</td>
<td>Control</td>
<td>PO</td>
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<tr>
<td></td>
<td>(n = 6)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
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<tr>
<td>HR (mmHg)</td>
<td>112 ± 4</td>
<td>284 ± 64**</td>
<td>157 ± 5</td>
<td>227 ± 12***</td>
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<tr>
<td>Diastolic (mmHg)</td>
<td>77.0 ± 1.8</td>
<td>112 ± 30</td>
<td>109 ± 2</td>
<td>93 ± 5**</td>
</tr>
<tr>
<td>Systolic pressure gradient (mmHg)</td>
<td>−3.4 ± 2.7</td>
<td>147 ± 57**</td>
<td>10.0 ± 9.5</td>
<td>103 ± 13***</td>
</tr>
<tr>
<td>LV developed pressure (mmHg)</td>
<td>110 ± 9</td>
<td>219 ± 16***</td>
<td>126 ± 5</td>
<td>232 ± 7***</td>
</tr>
<tr>
<td>Heart rate (1/s)</td>
<td>269 ± 17</td>
<td>315 ± 19</td>
<td>243 ± 6</td>
<td>254 ± 4</td>
</tr>
<tr>
<td>dp/dt max (indexed)</td>
<td>31.2 ± 2.6</td>
<td>23.9 ± 0.9</td>
<td>30.5 ± 1.8</td>
<td>19.7 ± 0.4***</td>
</tr>
<tr>
<td>dp/dt min (indexed)</td>
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<td>−19.5 ± 1.2</td>
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Data are mean ± SEM. dp/dt max and dp/dt min are indexed to left ventricular developed pressure; PO, pressure overload.

Table 3 Pressure and contractile measurements as performed by Millar catheter

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<th>10 Weeks</th>
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<td>−24.6 ± 1.5</td>
<td>−19.5 ± 1.2</td>
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Data are mean ± SEM. dp/dt max and dp/dt min are indexed to left ventricular developed pressure; PO, pressure overload.

Substrate oxidation and heart failure in rats
3.2 Impaired fatty acid oxidation precedes the onset of heart failure

Figure 3 shows rates of fatty acid (A) and glucose oxidation (B) of isolated hearts as well as the ratio of glucose to fatty acid oxidation (C), cardiac power (D), and cardiac power as a function of ATP production calculated from exogenous substrate oxidation (E). Unexpectedly, fatty acid oxidation was dramatically reduced already after 2 weeks of pressure overload and remained low thereafter. These findings were supported by reduced fatty acid oxidation gene expression, which showed a continuous decrease during pressure overload (Table 4). Pressure overload was associated with normal glucose oxidation during compensated hypertrophy (2 weeks) followed by a clear trend towards reduced glucose oxidation (significant at 20 weeks, \( P < 0.05 \)). Interestingly, the ratio of glucose to fatty acid oxidation showed an increase at all time points, illustrating the expected shift towards greater reliance on glucose oxidation. However, this ratio was elevated despite a decrease in both oxidation rates in three of the four time points. Cardiac power was reduced in all hearts with pressure overload, although hearts performed closer to normal after 2 weeks. The relation of power to calculated ATP production illustrates an almost normal relation 2 weeks after banding and significantly greater need of ATP per amount of power generated in all other groups.

![Figure 2](image-url)

**Figure 2** Lung-to-body weight index of animal subjected for 2, 6, 10, and 20 weeks to pressure overload compared with control animals. Data are mean \( \pm \) SEM; \( * \) \( P < 0.05 \), \( ** \) \( P < 0.01 \) numbers in the columns indicate group size (\( n \)).

3.3 Markers of heart failure parallel the reduction in fatty acid oxidation

Table 4 shows the expression of recognized markers of heart failure. Atrial natriuretic factor and brain natriuretic protein were increased after 2 weeks of pressure overload and remained high thereafter. There was no correlation with clinical presentation or the in vivo assessment of function. However, the increase in heart failure markers paralleled the decrease in fatty acid oxidation. The same was true for the myosin heavy chain isoforms. \( \alpha \)-MHC expression was decreased after banding already after 2 weeks, while \( \beta \)-MHC was increased. The ratio of \( \alpha \)/\( \beta \)-MHC reflects the known shift to the \( \beta \)-isoform in hypertrophy.

The significant changes in substrate oxidation rates in the isolated heart at times when EF and clinical presentation was normal (2, 6, 10 weeks of pressure overload) moved us to further investigate the oxidative capacity of isolated mitochondria.

3.4 Impairment of mitochondrial respiratory capacity parallels the impairment in EF

In order to gather information on the activity of the respiratory chain and its coupling to ATP production, we isolated cardiac mitochondria by differential centrifugation. Figure 4 shows maximal respiratory capacity and the ADP/O ratios of mitochondria isolated from

![Table 4](image-url)

**Table 4** Heart failure markers and expression of key genes of fatty acid oxidation in rats subjected to pressure overload

<table>
<thead>
<tr>
<th></th>
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<th>6 Weeks</th>
<th>10 Weeks</th>
<th>20 Weeks</th>
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<tr>
<td></td>
<td>Control ( (n = 7) )</td>
<td>PO ( (n = 6) )</td>
<td>Control ( (n = 7) )</td>
<td>PO ( (n = 7) )</td>
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<tr>
<td>ANF/ANP</td>
<td>0.22 \pm 0.04</td>
<td>2.25 \pm 0.12**</td>
<td>0.30 \pm 0.03</td>
<td>3.76 \pm 0.29***</td>
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<td>BNP</td>
<td>0.52 \pm 0.07</td>
<td>1.19 \pm 0.15***</td>
<td>0.67 \pm 0.19</td>
<td>1.73 \pm 0.17***</td>
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<tr>
<td>( \alpha )-MHC</td>
<td>1.07 \pm 0.32</td>
<td>0.21 \pm 0.04*</td>
<td>1.49 \pm 0.27</td>
<td>0.21 \pm 0.02***</td>
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<tr>
<td>( \beta )-MHC</td>
<td>0.19 \pm 0.08</td>
<td>0.59 \pm 0.13*</td>
<td>0.38 \pm 0.06</td>
<td>0.98 \pm 0.15**</td>
</tr>
<tr>
<td>( \alpha )/( \beta )-MHC</td>
<td>13.76 \pm 4.70</td>
<td>0.39 \pm 0.11*</td>
<td>3.96 \pm 0.37</td>
<td>0.24 \pm 0.04***</td>
</tr>
<tr>
<td>PPAR- ( \alpha )</td>
<td>0.74 \pm 0.08</td>
<td>0.55 \pm 0.05</td>
<td>0.87 \pm 0.13</td>
<td>0.59 \pm 0.06</td>
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<tr>
<td>FABP</td>
<td>0.40 \pm 0.07</td>
<td>0.50 \pm 0.18</td>
<td>1.13 \pm 0.27</td>
<td>0.62 \pm 0.15</td>
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<tr>
<td>FAT</td>
<td>0.51 \pm 0.18</td>
<td>0.27 \pm 0.14</td>
<td>0.53 \pm 0.05</td>
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</tr>
<tr>
<td>LCAD</td>
<td>0.51 \pm 0.08</td>
<td>0.28 \pm 0.05*</td>
<td>0.90 \pm 0.14</td>
<td>0.40 \pm 0.03**</td>
</tr>
<tr>
<td>MCAD</td>
<td>0.41 \pm 0.10</td>
<td>0.22 \pm 0.08</td>
<td>0.78 \pm 0.11</td>
<td>0.35 \pm 0.07**</td>
</tr>
<tr>
<td>mCPT1</td>
<td>0.47 \pm 0.10</td>
<td>0.33 \pm 0.04</td>
<td>0.88 \pm 0.14</td>
<td>0.49 \pm 0.08*</td>
</tr>
</tbody>
</table>

Relative mRNA expression normalized to S29. Data are mean \( \pm \) SEM. mRNA expression of ANF/ANP, atrial natriuretic factor/protein; \( \alpha \)-MHC, \( \alpha \)-myosin heavy chain; \( \beta \)-MHC, \( \beta \)-myosin heavy chain; PO, pressure overload. \( * \) \( P < 0.05 \), \( ** \) \( P < 0.01 \), \( *** \) \( P < 0.001 \); compared with age-matched controls after 2, 6, 10, 20 weeks of pressure overload by aortic constriction.
normal and pressure-overloaded rats at the four time points. Surprisingly, maximal respiratory capacity (state 3 respiration) was greater than control in the first 6 weeks of pressure overload, normal after 10 weeks, and significantly reduced after 20 weeks. Interestingly the decrease in respiratory capacity after 20 weeks paralleled the observed decrease in EF by echocardiography.

4. Discussion
Pressure overload-induced impairment in fatty acid oxidation precedes the onset of congestive heart failure in vivo but mitochondrial respiratory capacity is maintained until the EF decreases. These temporal relations suggest a tight link of impaired substrate oxidation capacity in the development of heart failure and contractile dysfunction and may imply therapeutic and prognostic value.

Others have demonstrated changes in substrate oxidation and energy metabolism in heart failure. Lipid oxidation was shown to be reduced in early heart failure in vivo. However, the question of a causal role of alterations in myocardial metabolism has been controversial for decades, specifically, because it has not been possible to determine whether the metabolic alterations have been a primary or an epi-phenomenon. The usual technique to refer to genetically altered mouse models has helped to identify metabolic mechanisms causing contractile dysfunction, including defects in glucose uptake, fatty acid oxidation, or mitochondrial biogenesis, but the role of metabolic changes in wild-type hearts developing heart failure is still not clear. We show here in a ‘wild-type’ rat

Figure 3 Fatty acid (A) and glucose oxidation (B) of isolated working hearts from rats subjected to 2, 6, 10, or 20 weeks of pressure overload compared with control, as well as the ratio of glucose to fatty acid oxidation (C), cardiac power (D), and cardiac power as a function of calculated ATP production from exogenous substrates (E). Data are mean ± SEM; *P < 0.05, **P < 0.01, ***P < 0.001 numbers in the columns indicate group size (n).
model that significant changes in oxidative substrate metabolism precede the onset of contractile dysfunction and heart failure in vivo. We further detected that respiratory capacity is also impaired but not until late, and that this decrease correlates with a significant impairment in EF in vivo. These time courses of events provide support for a primary defect in oxidative substrate metabolism, specifically fatty acid oxidation, and the secondary onset of heart failure and contractile dysfunction.

Aside from the metabolic changes, the assessment of contractile function both in vivo and ex vivo, the lung-to-body weight ratios, and the late presence of clinical heart failure symptoms suggest a continuous development of heart failure finally associated with abnormal EF. This end stage is reached through a state of compensated hypertrophy and one of the heart failures with normal EF in this animal model. While there is no classification available in animals thus far, such a classification would be consistent with the clinical definition of heart failure as suggested by the European Society of Cardiology.40 It finds further support by Borlaug et al.,41 showing reduced power at normal EF in patients with heart failure. These correlations underscore the relevance of our pressure overload model.

However, our classification is based on the measurements of lung weights, the assessment of pleural effusions as well as invasive pressure measurements. In practice, these parameters are usually not readily available and contractile function is assessed by echocardiography. Here it is interesting to note that the EF and FS were reduced much later than contractile function when assessed by Millar pressure measurements or by cardiac power ex vivo. Thus, echocardiography may be the method most frequently used in humans to screen for cardiac function, our findings support the notion that it is not necessarily the most sensitive method to detect systolic dysfunction, specifically if measurements are based on two-dimensional echocardiography.40

While echocardiography showed evidence for contractile dysfunction only after 20 weeks, indexed dp/dt was reduced already after 6 weeks. It is interesting to note that the decrease in dp/dt correlates with the onset of heart failure as indicated by the increase in lung-to-body weight ratios. Thus, echocardiography was not able to determine the onset of heart failure. In animal studies, the determination of the onset of heart failure is even less clear. Nowadays, LVEDD and the EF or FS are most frequently used for the diagnosis of contractile dysfunction, but reports about studies on echocardiographic determination of the severity of cardiac dysfunction in pressure overload models are limited. The echocardiographic detection of contractile function did also not match the assessment of invasive dp/dt, which presented a certain correlation to the heart failure signs observed. Interestingly, the ex vivo assessment of cardiac power was decreased even before any of the in vivo parameters indicated contractile dysfunction. While this observation may be interpreted as potential predictor of heart failure, it may be criticized for methodological reasons. It is conceivable that the pressure-overloaded hearts are more sensitive to myocardial stunning during the preparation process before perfusion. It could be criticized for the loading conditions, which, at least for the pressure-overloaded hearts, are different from the in vivo situation. The afterload was set at 74 mmHg (100 cmH2O) reflecting the coronary per- fusion pressure. This pressure is lower than coronary perfusion pressure in the pressure-overloaded animals in vivo, where the aortic constriction is performed in the aortic arch. However, contractile function of these hearts ex vivo was so poor that they barely managed to generate the afterload column of 100 cmH2O. Further elevations of the afterload in order to increase coronary perfusion pressure would therefore have been useless since poor cardiac output would not have been able to increase the fluid meniscus. Since coronary flow was not much different than in control hearts, we deduce that insufficient coronary flow did not contribute artificially to the poor function in the isolated heart system.

From a substrate oxidation perspective, the results suggest that pressure overload-associated decreases in substrate oxidation may predict the development of clinically relevant failure. In the introduction, we already described the concept of Neubauer suggesting a link between reduced substrate oxidation and contractile dysfunction. According to the diagram shown by Neubauer, the gradual decline of substrate oxidative capacity eventually leads to dysfunction and failure. Indeed, we see substantial impairments in both glucose and fatty acid oxidation and the timing of our findings would support a gradual decline. In summary, we suggest that the observed temporal sequence of events is strong argument for a tight link between fatty acid oxidation decline and progression to contractile dysfunction. Since fatty acid oxidation rates can be estimated by [11C]-palmitate

![Figure 4](https://academic.oup.com/cardiovascres/article-abstract/86/3/461/315777) Maximal respiratory capacity (A) and ADP/O ratio (B) of cardiac mitochondria with glutamate as substrate and ADP as stimulant. Data are mean ± SEM: \( \# p < 0.05, \# \# p < 0.01, \# \# \# p < 0.001 \) numbers in the columns indicate group size (n).
Substrate oxidation and heart failure in rats

uptake by positron emission tomography, the relevance may be assessed in humans.

The presented ratios of glucose to fatty acid oxidation would even support the conclusions of a substrate switch to glucose as an oxidative substrate. However, there is no significant increase in glucose oxidation at any time point. Thus, both oxidation rates decreased, excluding the possibility of a ‘shift’. The impairment of fatty acid oxidation is just greater than that of glucose oxidation. Interestingly, in the setting of heart failure, most described substrate switches are indeed mainly based on decreased fatty acid oxidation and not on increased glucose oxidation. The only study actually measuring glucose oxidation in failing hearts is the one by Osorio et al. Glucose oxidation in their study was measured by [14C]-glucose, but a true quantification was impossible because coronary flow was based on estimation. Thus our present study is the first to truly quantify fatty acid oxidation and glucose oxidation at sequential time points during the development of heart failure and contractile dysfunction. In contrast to a common belief, there is no evidence for increased rates of glucose oxidation at any time point. The well-described substrate switch is therefore a consequence of a greater reduction in fatty acid oxidation. Another aspect that needs to be addressed in this context is that of efficiency.

The Abel laboratory has demonstrated significant decreases in cardiac efficiency in diabetes. A similar mechanism is conceivable for heart failure, but the evidence is still wanting. Since we did not determine oxygen consumption, we are not able to present efficiency data. But we calculated the amount of ATP production from endogenous substrates. Our respiratory capacity and the ADP/O ratios of isolated mitochondria showed decreased respiratory rate and unchanged ADP/O ratios after 20 weeks of pressure overload. Since the ADP/O ratio was not altered, one may assume that the calculated rates of ATP production can be compared with controls. Thus, our present results suggest that more ATP is needed for a given amount of work in hearts with long standing pressure overload compared with controls. These findings provide indirect support for the decrease in efficiency in heart failure but also suggest that a limitation in ATP production may not be the only reason for contractile dysfunction in our model, since more ATP is needed per amount of power in heart failure than in controls. This question warrants further investigations.

5. Conclusions

We conclude that impairment of substrate oxidation exacerbates the evidence for heart failure by echocardiography in vivo. We suggest that quantification of substrate utilization has predictive value for the diagnosis and potential treatment of heart failure. We also advise caution in staging pressure overload heart failure by echocardiography or other commonly used markers for heart failure done in animal models.

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