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ORIGINAL RESEARCH ARTICLE

CARDIOKIN1: Computational Assessment of Myocardial Metabolic Capability in Healthy Controls and Patients With Valve Diseases

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BACKGROUND: Many heart diseases can result in reduced pumping capacity of the heart muscle. A mismatch between ATP demand and ATP production of cardiomyocytes is one of the possible causes. Assessment of the relation between myocardial ATP production (MV_{ATP}) and cardiac workload is important for better understanding disease development and choice of nutritional or pharmacologic treatment strategies. Because there is no method for measuring MV_{ATP} in vivo, the use of physiology-based metabolic models in conjunction with protein abundance data is an attractive approach.

METHOD: We developed a comprehensive kinetic model of cardiac energy metabolism (CARDIOKIN1) that recapitulates numerous experimental findings on cardiac metabolism obtained with isolated cardiomyocytes, perfused animal hearts, and in vivo studies with humans. We used the model to assess the energy status of the left ventricle of healthy participants and patients with aortic stenosis and mitral valve insufficiency. Maximal enzyme activities were individually scaled by means of protein abundances in left ventricle tissue samples. The energy status of the left ventricle was quantified by the ATP consumption at rest (MV_{ATP}[rest]), at maximal workload (MV_{ATP}[max]), and by the myocardial ATP production reserve, representing the span between MV_{ATP}(rest) and MV_{ATP}(max).

RESULTS: Compared with controls, in both groups of patients, MV_{ATP}(rest) was increased and MV_{ATP}(max) was decreased, resulting in a decreased myocardial ATP production reserve, although all patients had preserved ejection fraction. The variance of the energetic status was high, ranging from decreased to normal values. In both patient groups, the energetic status was tightly associated with mechanic energy demand. A decrease of MV_{ATP}(max) was associated with a decrease of the cardiac output, indicating that cardiac functionality and energetic performance of the ventricle are closely coupled.

CONCLUSIONS: Our analysis suggests that the ATP-producing capacity of the left ventricle of patients with valvular dysfunction is generally diminished and correlates positively with mechanical energy demand and cardiac output. However, large differences exist in the energetic state of the myocardium even in patients with similar clinical or image-based markers of hypertrophy and pump function.

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Key Words: energy metabolism ■ heart valve diseases ■ mathematical model ■ metabolism ■ proteomics

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Clinical Perspective

What Is New?

- We developed CARDIOKIN1, a novel comprehensive molecular-resolved kinetic model of central cardiac metabolism.
- CARDIOKIN1 enables new insights into regulatory principles of cardiac metabolism and allows patient-specific evaluation of metabolic capacities on the basis of proteomics data.
- We applied the method in patients who had significant change in cardiac workload owing to aortic and mitral valve diseases.

What Are the Clinical Implications?

- Common heart valve diseases, such as aortic stenosis and mitral regurgitation, lead to significant impairment in cardiac metabolic capacity.
- Metabolic capacities have a significant correlation with biomechanical measures such as myocardial power and cardiac output but can vary considerably between individual patients, contributing to understanding why some patients develop heart failure over time, whereas others with similar hemodynamic conditions do not.
- Individual metabolic capacities are associated with postoperative outcomes and may be a helpful prognostic marker.

Nonstandard Abbreviations and Acronyms

AS	aortic stenosis
BCAA	branched-chain amino acid
BP	blood pressure
FFA	free fatty acid
HR	heart rate
KB	ketone body
LV	left ventricle
MAPR	myocardial ATP production reserve
MVI	mitral valve insufficiency
MV_{ATP}	myocardial ATP production capacity
MV_{ATP}(max)	myocardial ATP consumption at maximal workload
MV_{ATP}(rest)	myocardial ATP consumption at rest
MVo_2	myocardial oxygen consumption
NYHA	New York Heart Association

In recent years, numerous studies have firmly established metabolic derangement as a cardinal feature of the pathophysiology of heart diseases.[1–5] Although changes in cardiac metabolism are understood to be an underlying component in almost all cardiac myopathies, the potential contribution of myocardial energy metabolism to the reduction of cardiac performance is not fully understood.[6] As early as 1939, Herrmann and Decherd Jr[7] proposed that the failing heart is an energy-starved engine that has run out of fuel. Several decades later, this concept has been reaffirmed,[3] primarily in light of findings that in the failing heart, the gene expression of key proteins involved in cardiac energy metabolism is downregulated (see examples)[8,9] and so is the level of central metabolic enzymes (eg, creatine kinase)[10] and of cardiac energy-rich phosphates (ATP, CrP), as established by ^{31}P magnetic resonance spectroscopy in vivo.[11,12] Although these findings are suggestive for a mismatch between ATP demand and ATP supply, they do not permit assessment of the degree of mismatch.

A common cause of cardiac dysfunction is valvular heart disease. The most frequent types of valve disease are aortic stenosis (AS) and mitral valve insufficiency (MVI), which expose the heart to long-term pressure or volume overload, respectively. Chronic pressure or volume overload trigger cardiac remodeling, leading to specific forms of myocardial hypertrophy. Pressure overload typically results in eccentric myocardial hypertrophy with wall thickness increase, whereas volume overload is dominated by concentric forms with increase of ventricular chamber volumes. Some patients tolerate the overload condition well for years, whereas others quickly change from compensated to decompensated heart failure despite similar cardiac pump function characteristics. Therefore, it is of particular importance in the clinic to be able to predict the risk for transition from compensated to decompensated heart failure as accurately as possible. This would include better knowledge about the metabolic status of the myocardium in heart diseases. Using cardiac magnetic resonance imaging and ^{31}P magnetic resonance spectroscopy, Peterzan et al.[13] addressed whether reduced delivery of ATP from mitochondria to the myosin ATPase by the creatine kinase shuttle may explain why some (but not all) patients with severe AS develop otherwise unexplained reduced left ventricular (LV) ejection fraction. Their data do not provide evidence for a significant difference in the creatine kinase flux of patients with cardiac dysfunction. This finding raises the question whether it is the gradual reduction in the myocardial ATP production capacity (MV_{ATP}) that parallels the deterioration of the LV systolic function that is at issue. The problem with testing this hypothesis is that no method is available to measure the ATP production rate MV_{ATP} in vivo. The aim of this work was to develop a method allowing assessment of the capability of the LV to increase MV_{ATP} in response to an increase in ATP demand.

On the basis of our network reconstruction of the metabolic network of the cardiomyocyte[14] and previous modeling work on metabolic subsystems,[15–18] we established a complex physiology-based mathematical model of the myocardial energy metabolism. The model encompasses all pathways along which the possible energy-

delivering substrates glucose, long-chain fatty acids, ketone bodies (KBs), acetate, and branched-chain amino acids (BCAAs) are used. As in our previous model-based studies on liver metabolism,[19] we used the proteomics-based abundance of metabolic enzymes in cardiac tissue to generate individualized metabolic models of cardiac energy metabolism. Applying this approach to the LV of controls and patients with MVI and AS, we tested the hypothesis that despite overall preserved systolic function, the ATP production capacity of the LV is reduced and correlates positively with mechanic energy demand and cardiac output.

METHODS

The analytic methods (model equations) are available in the Supplemental Materials for other researchers for purposes of replicating the procedure. Patients' proteomics data will not be made available to other researchers.

Patient Characteristics

We investigated 75 human LV myocardial biopsies. Myocardial samples from the LV septum were collected during surgical aortic or mitral valve replacement from 41 patients with AS and 17 patients with MVI. Patient characteristics are described in the Table. For the controls (n = 17), samples were taken from 44 ≥15-year-old donors without cardiac diseases but whose hearts were not used for transplantation. All samples were frozen immediately in liquid nitrogen until further processing.

The study protocol was in agreement with the principles outlined in the Declaration of Helsinki and was approved by the Medical Ethics Review Committee. All patients gave written informed consent before inclusion.

Quantitative Proteomics of Tissue Samples

LV septum biopsies were extracted at time of surgery, frozen directly in liquid nitrogen, and kept at −80°C. For protein extraction, biopsies were lysed in 200 µL lysis buffer (2% SDS, 50 mM ammonium bicarbonate buffer, and EDTA-free protease inhibitor cocktail [Complete; Roche]) and homogenized using FastPrep-24 5G Homogenizer (MP Biomedicals; 10 cycles of 20 seconds with 5-second pause). After heating for 5 minutes at 95°C and 5 freeze–thaw cycles, Benzonase (25 U; Merck) was added for 30 minutes and lysates were clarified by centrifugation at 16 000 *g* for 40 minutes. A total of 100 µg protein per sample was processed further using the SP3 clean-up and digestion protocol as previously described.[20] Briefly, each sample was reduced with 10 mM dithiothreitol (Sigma), followed by alkylation with 40 mM chloroacetamide (Sigma) and quenching with 20 mM dithiothreitol (Sigma). Next, 1 mg beads and acetonitrile (70% final concentration) were added to each sample and after 20 minutes of incubation, bead-bound protein was washed with 70% ethanol and 100% acetonitrile. Then, 2 µg sequence-grade Trypsin (Promega) and 2 µg LysC (Wako) in 50 mM HEPES (pH 8) were added. After overnight incubation at 37°C, peptides were collected, acidified with trifluoroacetic acid, and cleaned up using the StageTips protocol.[21]

Heart Reference Sample for Matching Library

A peptide mix for each experimental group (control, AS, and MVI) was generated by collecting 10 µg peptides from each individual sample belonging to the corresponding group. Equal peptide amounts from each group mixture were combined, fractionated into 96 fractions by basic reversed-phase chromatography (XBridge C18 4.6 mm × 250 mm column [Waters, 3.5 µm bead size]; Agilent 1290 high-performance liquid chromatography instrument; 90 minutes gradient; flow rate 1 mL/min), and pooled into 26 equal interval fractions for further analyses.

Liquid Chromatography With Tandem Mass Spectrometry Analyses

Peptides were separated by reversed-phase chromatography (20 cm column; 75 µm inner diameter, ReproSil-Pur C18-AQ; 1.9 µm, Dr Maisch GmbH) using a 200-minute gradient (flow rate 250 nL/min) on a high-performance liquid chromatography system (ThermoScientific). Measurements were performed on an Orbitrap Fusion (individual samples) or Q Exactive HF-X Orbitrap instrument (reference sample; ThermoScientific) using data-dependent acquisition. Each sample was measured twice and replicates were joined for data analysis. Data were analyzed using MaxQuant (v1.6.2.6) and a decoy human UniProt database (2019-01).[22] Variable modifications of oxidation (M), N-terminal acetylation, deamidation (N, Q), and fixed modification of carbamidomethyl cysteine were selected. The false discovery rate was set to 1% for peptide and protein identification. Unique and razor peptides were considered for quantification. Match between runs and label-free quantification algorithm were applied.

Description of the Mathematical Model (CARDIOKIN1)

For quantification of the metabolic changes caused by the abundance changes of metabolic enzymes, we developed a mathematical model of the cardiac energy metabolism, which comprises all pathways involved in the catabolism of the energy-delivering substrates glucose, lactate, fatty acids, KBs, and BCAAs, as well as the synthesis of endogenous energy stores (glycogen, triacylglycerol; Figure 1). The model also takes into account the short-term regulation of metabolic enzymes and transporters by the hormones insulin and catecholamines and key electrophysiologic processes at the inner mitochondrial membrane including generation of the proton gradient by the respiratory chain, synthesis of ATP by FoF1-ATPase, and membrane transport of various ions.

The time course of model variables (concentration of metabolites and ions) is governed by first-order differential equations. Time variations of small ions are modeled by kinetic equations of the Goldman-Hodgkin-Katz type as used in our previous work.[23] The rate laws for enzymes and membrane transporters were either taken from the literature or constructed on the basis of published experimental data for the mammalian heart. The used rate laws take into account the regulation of enzymes and transporters by reaction substrates and products, allosteric effectors, and reversible phosphorylation as compiled by biochemists during decades of enzymatic research. Supplement S1 contains all kinetic equations and model parameters sorted by individual pathways. For each kinetic parameter value, we indicated the experimental source.

Table. Patient Characteristics

Characteristics and preoperative function measures	AS	MVI	*P* value
Age at surgery, y	68±9	60±14	0.032
BMI	28±4	27±3	0.343
Female sex	23 (56)	6 (35)	0.414
NYHA functional classification, stage I/II/III/IV	5/17/15/1	2/7/6/2	0.593
Systolic blood pressure, mm Hg	140±19	131±16	0.123
Diastolic blood pressure, mm Hg	74±11	75±13	0.675
EDVI, mL/m^2	73±17	108±34	<0.001
ESVI, mL/m^2	30±11	40±14	0.015
EF, %	60±7	62±9	0.048
CI, L/min/m^2	3±1	5±2	<0.001
CO, L/min	6±2	9±4	<0.001
Internal myocardial power	13±7	13±5	1
Myocardial mass (indexed), g/m^2	71±20	67±15	0.484
Mean pressure gradient aortic valve, mm Hg	56±15	4±8	<0.001
Mitral valve insufficiency, none/mild over moderate to severe	41/0/0	0/10/7	<0.001
Aortic valve insufficiency, none/mild over moderate to severe	36/5/0	17/0/0	0.321
Serum creatinine, mg/dL	0.91±0.15	1.0±0.20	0.065
Hypertension	27 (66)	11 (65)	0.826
Dyslipidemia	8 (20)	3 (18)	0.839
Diabetes type 2	7 (17)	2 (12)	0.913
Coronary artery disease	1 (2)	2 (12)	0.419
Atrial fibrillation, paroxysmal	2 (5)	2 (12)	0.709
Atrial fibrillation, permanent	0 (0)	2 (12)	0.149
Medication ACE inhibitor	15 (37)	5 (29)	0.826
Medication β-blocker	20 (49)	10 (59)	0.683
Medication diuretics	12 (29)	5 (29)	0.760

Data are presented as total number (%) in case of categorical values and as mean±SD in case of numeric values. Significance of parameter differences between the 2 patient groups is given as *P* values. ACE indicates angiotensin-converting enzyme; AS, aortic stenosis; BMI, body mass index; CI, cardiac index; CO, cardiac output; EDVI, end-diastolic volume index; EF, ejection fraction; ESVI, end-systolic volume index; MVI, mitral valve insufficiency; and NYHA, New York Heart Association.

Model Calibration for Individual Hearts

Among the kinetic parameters of the enzymatic rate laws (binding constants of ligands, cooperativity indices), only V_{max}, representing the maximal activity of an enzyme/transporter, may vary among individual LVs as the value of this parameter is proportional to the enzyme abundance: $V_{max} = k_{cat} \cdot E$, with k_{cat} being the turnover rate of a single enzyme and E the enzyme concentration. Exploiting this simple relationship, we used the proteomics-derived protein profiles of enzymes and transporters for model calibration by computing the maximal activities (V_{max}) of the enzymes by the relation

$$V_{\max}^{subject} = V_{\max}^{normal} \frac{E^{subject}}{\langle E^{control} \rangle} \quad (1)$$

where $\langle E^{control} \rangle$ is the average protein intensity of the enzyme in the group of control hearts and $E^{subject}$ is the protein concentration of the enzyme in the individual (control or patient). The maximal activities $v_{\max}^{normal}$ of the reference model for the average normal heart were obtained by fitting of the model to experimental data (Table S2). Relation (1) follows from the fact that the maximal enzyme activity is proportional to the abundance of the protein.

Energetic Capacities of Controls and Patients With Valve Diseases

We used the model to compute the specific uptake rates of substrates and the specific ATP production rate at rest, MV_{ATP}(rest), and at maximal ATP workload, MV_{ATP}(max), for the LV of controls (n=17) and patients with MVI (n=17) or AS (n=41). As a third parameter to characterize the capacity of the LV to increase ATP production with increasing workload, we used the span between MV_{ATP}(max) and MV_{ATP}(rest), which we refer to as myocardial ATP production reserve (MAPR = MV_{ATP}[max] − MV_{ATP}[rest]). In the following, we distinguish among specific energy parameters MV_{ATP}(rest), MV_{ATP}(max), and MAPR quantifying the energetic capacity per mass unit of the LV (given in µmol/g/h) and total energy parameters tMV_{ATP}(rest), tMV_{ATP}(max), and tMAPR quantifying the energetic capacity of the LV (given in mmol/h; ie, tMV_{ATP}[rest] = MV_{ATP}[rest] × LV mass/1000).

The computations were performed for a normal postabsorptive state (overnight fast) characterized by the following metabolite and hormone concentrations: glucose 5.8 mM,[24] fatty acids 0.5 mM,[25] lactate 0.8 mM,[24] glutamine 0.5 mM,[26,27] valine 0.2 mM,[26,27] leucine 0.15 mM,[26,27] isosleucine 0.06 mM,[26,27] β-hydroxybutyrate 0.08 mM,[28,29] and acetoacetate 0.04 mM.[30] The concentration of catecholamines at rest was 0.75 nM[31,32] and increased with growing workload (see Supplement S2, including Figures S1–S4).

The myocardial ATP consumption of the stationary resting state, MV_{ATP}(rest), was chosen in a way that the computed myocardial oxygen consumption (MVo_2) was identical with the participant's MVo_2, which we estimated by the 2-factor approximation[33]

$$MV_{O2} = \gamma \cdot HR \cdot BP \quad (2)$$

using heart rate (HR) and peak systolic blood pressure (BP) and γ as a proportionality factor. Resting MVo_2 of normal hearts was found in the range of 0.8 to 1.2 mL/min/g.[2,4,34] Thus, with a mean MVo_2 of 0.1 mL/min/g, HR of 70/min, and normal BP of 125 mm, we set γ to 1.143×10^{-5} mL/mm Hg/g.

The metabolic response of the ventricle to an additional workload (pacing) was evaluated by computing the temporal changes of the metabolic state elicited by an increase of the ATP consumption rate above the resting value. The ATP consumption rate was modeled by a generic hyperbolic rate law

$$v_{ATP} = k_{load} \cdot \frac{ATP}{ATP + K_m} \quad (3)$$

(Supplement S1). The parameter k_{load} was stepwise increased until MV_{ATP} converged to the maximum: MV_{ATP}(max).

To evaluate the mechanical burden of the heart, we calculated the internal myocardial power, which describes the energy required for cardiac contraction for the individual hearts (see methods used in Lee et al[35]).

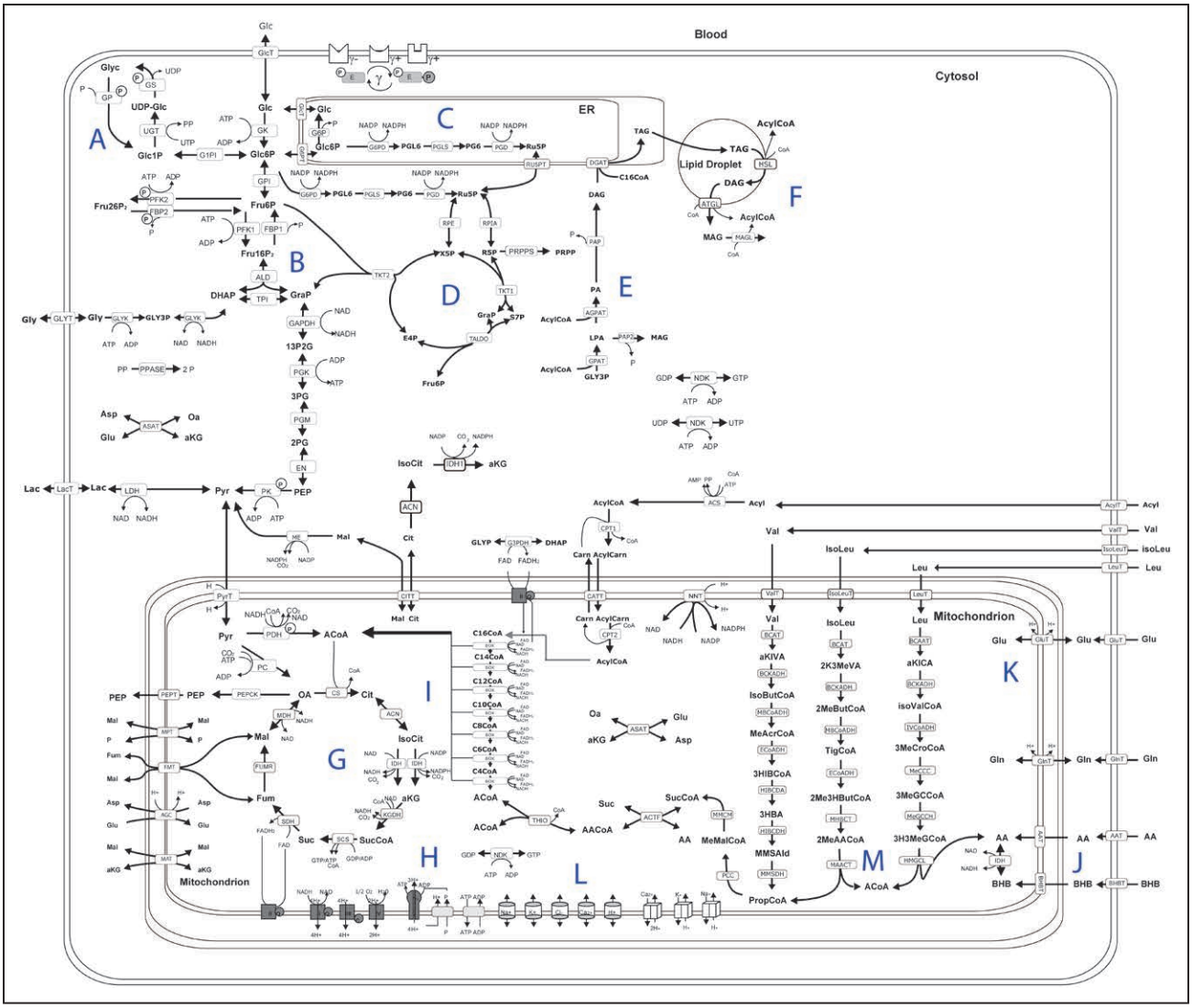

Figure 1. Reaction scheme of the metabolic model.
Arrows symbolize reactions and transport processes between compartments. **A**, Glycogen metabolism, (**B**) glycolysis, (**C**) oxidative pentose phosphate pathway in the endoplasmic reticulum and cytosol, (**D**) nonoxidative pentose phosphate pathway, (**E**) triglyceride synthesis, (**F**) synthesis and degradation of lipid droplets, (**G**) tricarbonic acid cycle, (**H**) respiratory chain and oxidative phosphorylation, (**I**) β-oxidation of fatty acids, (**J**) ketone body utilization, (**K**) glutamate metabolism, (**L**) mitochondrial electrophysiology (membrane transport of ions), and (**M**) utilization of branched-chain amino acids. Small cylinders and cubes symbolize ion channels and ion transporters. Double arrows indicate reversible reactions, which, according to the value of the thermodynamic equilibrium constant and cellular concentrations of their reactants, may proceed in both directions. Reactions are labeled by the short names of the catalyzing enzyme or membrane transporter given in the small boxes attached to the reactions arrow. Metabolites are denoted by their short names. Full names and kinetic rate laws of reaction rates are outlined in Supplement S1. Full names of metabolites and a comparison of experimentally determined and calculated cellular metabolite concentrations are given in Table S1.

All model simulations were performed using MATLAB, Release R2011b (The MathWorks, Inc).

RESULTS

Model Calibration for the Normal Heart

Except for the maximal enzyme activities (V_{max} values), which may vary owing to variable gene expression, the numeric values for all other parameters of the enzymatic rate laws were taken from reported kinetic studies of the isolated enzymes. Numeric values for the V_{max} values (Table S2) were estimated by the same procedure that was used for the calibration of our metabolic liver model[19]: calculated metabolite profiles and fluxes were adjusted to experimental data from independent experiments with perfused hearts and in vivo measurements whereas the metabolite concentrations were constrained to experimentally determined ranges (Table S1). Short-term regulation of key regulatory enzymes by the hormones insulin and catecholamines (epinephrine, norepinephrine) was included into the model by phenomenologic mathematical functions relating the enzyme's phosphorylation state and the abundance of the GLUT4 transporter in the

sarcolemma to the plasma concentrations of glucose (insulin) and the exercise level (catecholamines; Supplement S2). Model simulations, which correctly recapitulate metabolic measurements obtained with perfused hearts and in human in vivo studies, comprise glucose utilization at varying exogenous glucose concentrations; lactate utilization and lactate/O_2 ratio at varying exogenous lactate concentrations; utilization of free fatty acids (FFAs) at varying exogenous FFA concentrations; glucose utilization in response to varying exogenous concentration of FFAs (glucose–FFA competition); KB utilization at varying exogenous β-hydroxybutyrate concentrations; utilization rates of glucose, lactate, FFAs, KBs, and BCAA under postabsorptive resting conditions; and utilization rates of glucose, lactate, FFAs, KBs, and BCAAs at moderate pacing. Details of all validation simulations are given in Supplement S3 (including Figures S5–S10 and Tables S3–S8).

Figure 2 shows 2 model validations highlighting the good concordance of model predictions with experimental data. The examples demonstrate the ability of the heart to ensure cardiac functionality at varying cardiac workloads and varying plasma concentrations of energy substrates. In Figure 2A, the computed substrate uptake profile of the normal human heart is compared with the mean of experimental data taken from several in vivo studies.[36–43] At rest, lactate is utilized with the highest rate, followed by FFAs and KBs. Counted in moles ATP per moles substrate (textbook values: glucose 38, lactate 18, palmitate 138), FFAs represent the dominating energy source. At moderate pacing, the uptake of the carbohydrates is more than doubled, whereas the uptake of FFAs remains essentially unaltered. The energetic contribution of BCAAs was <1% at rest and pacing. Figure 2B shows the relationship between glucose uptake and plasma FFA concentration. The uptake rate of glucose is suppressed with increasing levels of plasma FFAs by inhibition of glucose uptake[44] ensuring the preferential utilization of fatty acids (Figure 2B).

Patient-Specific Model Calibration

For patient-specific model calibration, we used protein intensity profiles (defined through label-free quantification intensities; see Methods) of 17 control hearts, 41 patients with AS, and 17 patients with MVI. Using 2-dimensional liquid chromatography before tandem mass spectrometry analysis, we identified, in total, 9133 distinct protein groups, from which a subset of 321 proteins was used for model calibration.

Energetic Capacities of the LV of Controls and Patients With Valve Diseases

Figure 3 depicts the specific energetic parameters MV_{ATP}(rest), MV_{ATP}(max), and MAPR for each participant after 60-minute pacing. Compared with controls, the individual variations of these parameters were much larger for the 2 patient groups (see box plots in Figure 3B–3D). For patients with MVI, the mean value of the param-

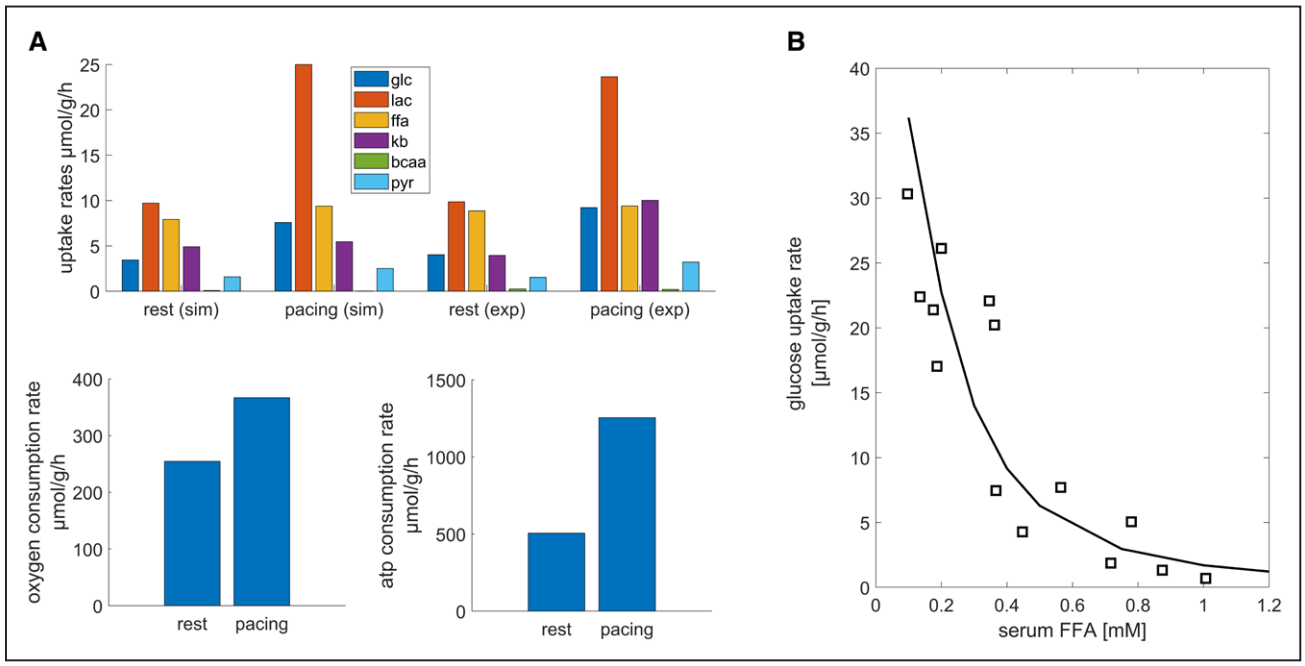

Figure 2. Simulated and measured myocardial substrate uptake rates in vivo.
A, Substrate uptake rates at rest and at moderate pacing (50% maxVo_2). (sim) Uptake rates were computed from reported extraction rates (1 – arterial concentration/concentration in coronary sinus) putting the coronary blood flow to 0.8 mL/min/g and heart weight to 300 grams. (exp) The data points represent the means of various experimental studies.[37,38,40–43,45] **B**, Dependence of the glucose uptake rate from the plasma concentration of free fatty acids (FFAs). The solid line represents model values; squares symbolize in vivo data taken from Nuutila et al.[44]

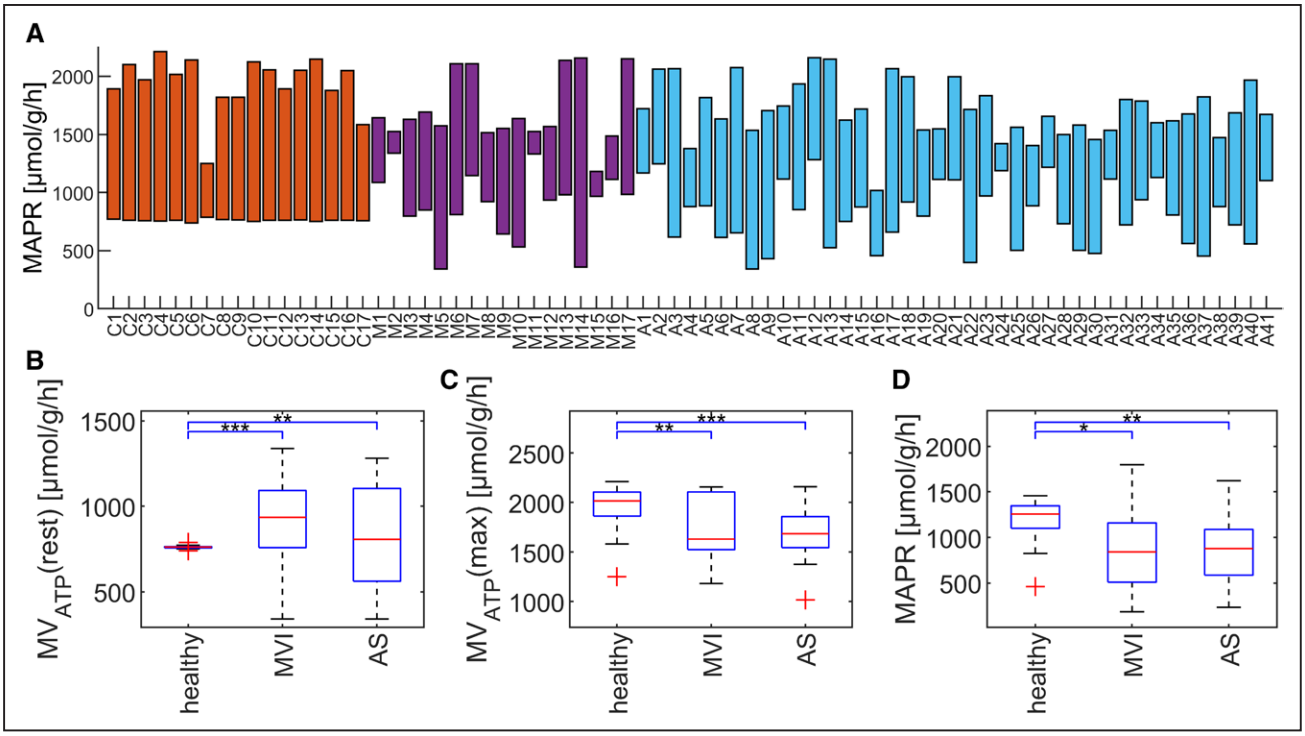

Figure 3. MV_{ATP}(rest) and MV_{ATP}(max) for controls and patients with mitral valve disease and aortic stenosis.
A, Bottom values of the bars refer to MV_{ATP}(rest); top values refer to MV_{ATP}(max). The bar length indicates the myocardial ATP production reserve (MAPR=MV_{ATP}[max] – MV_{ATP}[rest]). **B** through **D**, Box plots showing mean values, upper and lower quartiles, and total span of MV_{ATP}(rest), MV_{ATP}(max), and MAPR for controls and patients with mitral valve insufficiency (MVI) and aortic stenosis (AS). Significant differences between the patient groups are indicated by connecting brackets with asterisks giving the significance level (*P<0.05, **P<0.01, ***P<0.001). A Bonferroni correction was applied to account for multiple testing.

eter MV_{ATP}(rest) was significantly higher (890±292 vs 761±10 µmol/g/h), whereas MV_{ATP}(max) was significantly lower when compared with control values (1713±245 vs 1941±238 µmol/g/h; 2-sample Kolmogorov-Smirnov test). For patients with AS, the mean value of the parameter MV_{ATP}(rest) was also significantly higher (800±270 vs 761±10 µmol/g/h) and MV_{ATP}(max) was also significantly lower (1513±257 vs 1941±238 µmol/g/h). For both groups of patients, the parameter MAPR was on average significantly lower compared with the controls (826±448 in MVI and 904±340 in AS vs 1180±245 µmol/g/h). Hence, both groups of patients had on the average a reduced ATP production reserve, which was caused by increased MV_{ATP}(rest) and decreased MV_{ATP}(max).

Substrate Uptake of Patients at Rest and at Maximal Workload

Next, we investigated changes in the substrate preference of the LV accompanying altered metabolic capacity (Figure 4). In the resting state, the largest differences occurred for the uptake rates of glucose and lactate for patients with MVI. Glucose uptake was increased by >20%. At rest, there was a significant decrease in lactate utilization in patients with AS. In general, variances in substrate utilization rates were large, again pointing to individually differing metabolic phenotypes. The reduction in lactate utilization was significant also at maximal load in MVI and AS; glucose rates were significantly reduced only in patients with AS. Figure 4 also shows how the different substrates contribute to overall energy production. The contribution of fatty acids accounts for up to 2-thirds, whereas BCAAs always account for <1% of total energy expenditure and are therefore not shown.

Association of MV_{ATP} With Clinical Measures Evaluating the Mechanical Work and the Systolic Performance of the LV

In valve disease, the LV is exposed to chronic pressure load (AS) or volume overload (MVI). This results in a higher mechanical workload, which can be quantified by the surrogate internal myocardial power estimating the power of the LV required for cardiac contraction.[35] Our analysis provided evidence for a strong correlation between tMV_{ATP} at rest and at maximal pacing and the internal myocardial power (Figure 5). A significant correlation of the energy parameters has also been found with the cardiac output (Figure 5). Taken together, these findings demonstrate a close association between increased ATP production capacity, increased mechanical work of the pressure/volume overloaded LV, and cardiac output.

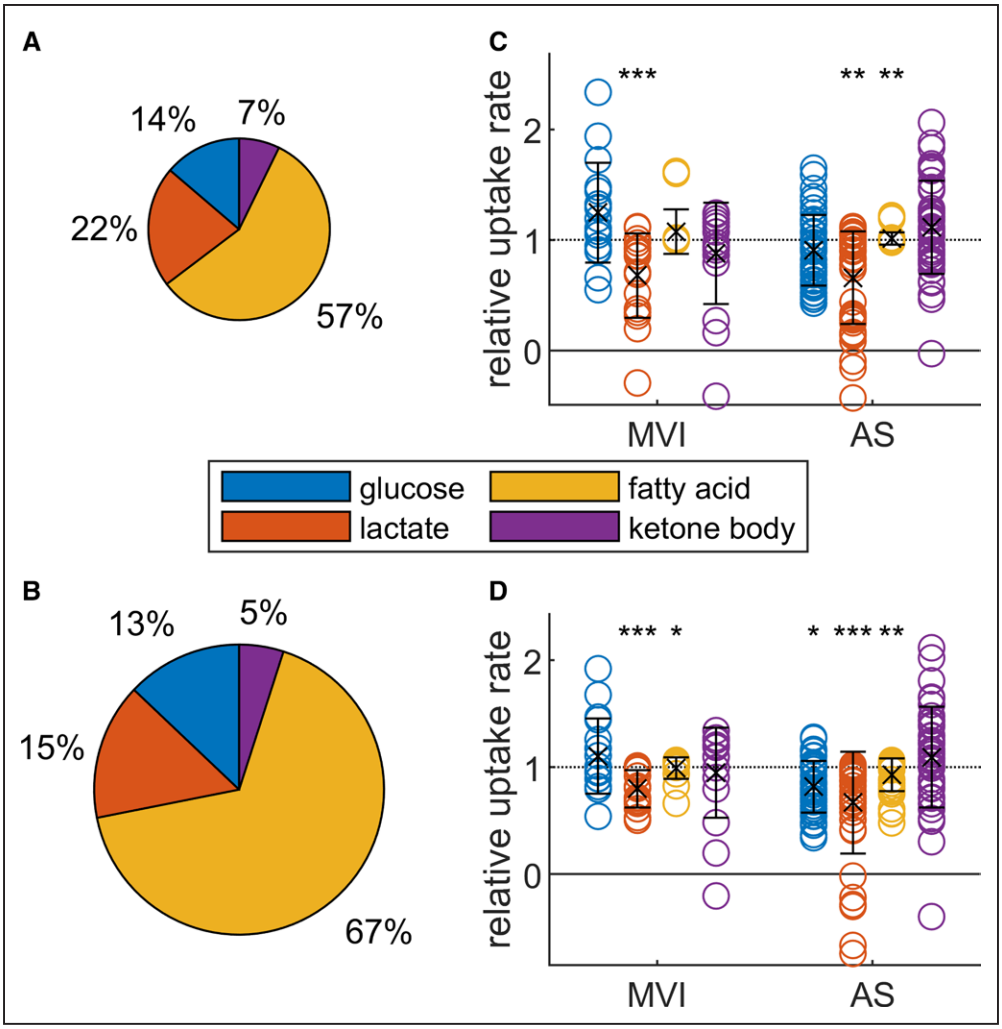

Figure 4. Contribution of energy-delivering substrates.
A and **B**, Relative contribution of the energy-delivering substrates to total energy expenditure at MV_{ATP}(rest) and MV_{ATP}(max) for the control group for 60 minutes pacing. Areas of the pie charts represent total energy expenditure. Changes of substrate uptake rates of patients with mitral valve insufficiency (MVI) or aortic stenosis (AS) relative to controls are shown at rest (**C**) and during maximal pacing (**D**). Plots show the relative change of substrate uptake rates of glucose (blue), lactate (orange), fatty acids (yellow), and ketone bodies (purple) for patients with MVI or AS during rest and at maximal ATP production rate after 60 minutes of pacing. Relative uptake rates are normalized to control values (ie, all control values are equal to 1). Significant changes from control are indicated by asterisks (*P<0.05, **P<0.01, ***P<0.001).

Metabolic Profiling of Individual Patients

Despite the general trend of the energy parameters in the patients' LV outlined in the preceding section, substantial differences in the metabolic profiles of individual patients occur. As an example, Figure 6 depicts the individual energetic profiles of 3 patients with AS with largely differing values of their cardiac energy parameters (Figure 3A). Patients A2 and A4 are characterized by impaired MAPR; patient A13 has a MAPR comparable to healthy hearts (Figure 3). The impaired MAPR of patient A2 results from an increased MV_{ATP}(rest) with a normal MV_{ATP}(max), whereas the impaired MAPR of patient A4 results from an increased MV_{ATP}(rest) and a decreased MV_{ATP}(max). Patient A13 with a normal MAPR has normal MV_{ATP}(rest) and normal MV_{ATP}(max). The individual alterations in the energetics of the LV are also associated with marked differences in substrate utilization rates. For example, whereas patient A13 has normal MV_{ATP}(rest), resting carbohydrate utilization rates (glucose and lactate) are strongly decreased and compensated by an increased KB utilization rate. This increased KB utilization is also maintained at MV_{ATP}(max) and is even more pronounced in patient A2. In contrast, patient A4 shows a decreased utilization rate for all substrates at MV_{ATP}(max).

Association of Preoperative Cardiac Metabolism With Postoperative Outcome

We also checked for a possible association between LV performance after valve surgery and the preoperative metabolic status of the LV. A univariate rank correlation analysis revealed a statistically significant association be-

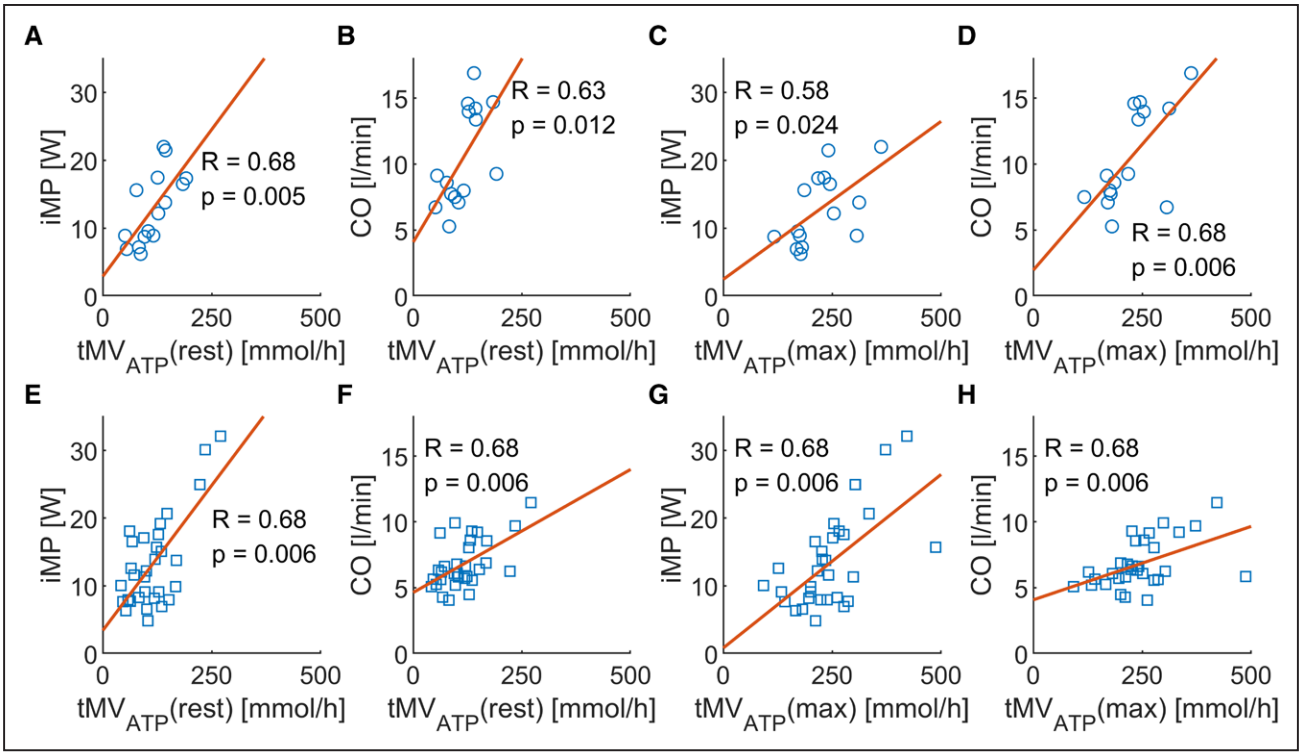

Figure 5. Correlation between tMV_{ATP}(rest) and tMV_{ATP}(max) and internal myocardial power as well as cardiac output for patients with mitral valve insufficiency or aortic stenosis.
A through **D,** Patients with mitral valve insufficiency (MVI). **E** through **H**, Patients with aortic stenosis (AS). iMP indicates internal myocardial power; and CO, cardiac output.

tween change in New York Heart Association (NYHA) functional classification (ΔNYHA = $NYHA_{postoperative}$ – $NYHA_{preoperative}$) and several metabolic capacities (Table S9), among them the maximal ATP production capacity in the postprandial state (P=0.02) and the maximal lactate uptake rate (P=0.01). This indicates that preoperative metabolic capability of the LV has an important effect on recovery and might be used as a prognostic marker in the future.

Statistical Analysis

For the patient characteristics in the Table, significance of parameter differences between AS and MVI groups was evaluated by means of 2-sided, 2-sample Wilcoxon-rank test in case of numeric data and χ^2 test with Yates continuity correction in case of categorical data.

Statistical significances between patient groups in Figure 3 and Figure 4 were evaluated by means of nonparametric Kolmogorov-Smirnov test. Statistical significance in Figure 3 was corrected for multiple testing by means of Bonferroni correction.

Correlations in Figure 5 are given as Pearson linear correlation coefficients with P values indicating statistical significance of the correlation computed by means of the Student t test.

Correlations between presurgery and postsurgery differences in NYHA Functional Classification and metabolic capacities (Table S9) were evaluated by calculation of Spearman rank correlation coefficients with P values indicating statistical significance of the correlation computed by means of the exact permutation test.

DISCUSSION

Novel Approach to Assess Myocardial ATP Producing Capacity

No method is available to measure MV_{ATP} in vivo. Invasive techniques, such as the determination of substrate extraction rates from coronary sinus, arterial concentration differences, or the oxidation rates of ^{14}C-labeled substrates from the rates of $^{14}CO_2$, have been applied in healthy people and patients with heart diseases.[36,37,46] However, such data cannot be directly converted into rates of ATP production. The same holds true for measurement of the myocardial oxygen consumption rate MVo_2 reflecting the overall myocardial oxidative metabolism. The MVo_2 does not capture the glycolytic ATP contribution, which is low under normoxic conditions but may increase 5-fold during development of heart failure[47] or even 20-fold during the transition from aerobic to anaerobic energy production.[48] Moreover, the ATP/O_2 ratio may change considerably with increasing workload owing to increasing cardiac preference for carbohydrates. This makes it difficult to convert O_2 consumption rates into ATP consumption rates. In addition, the maximal MVo_2 can be low because

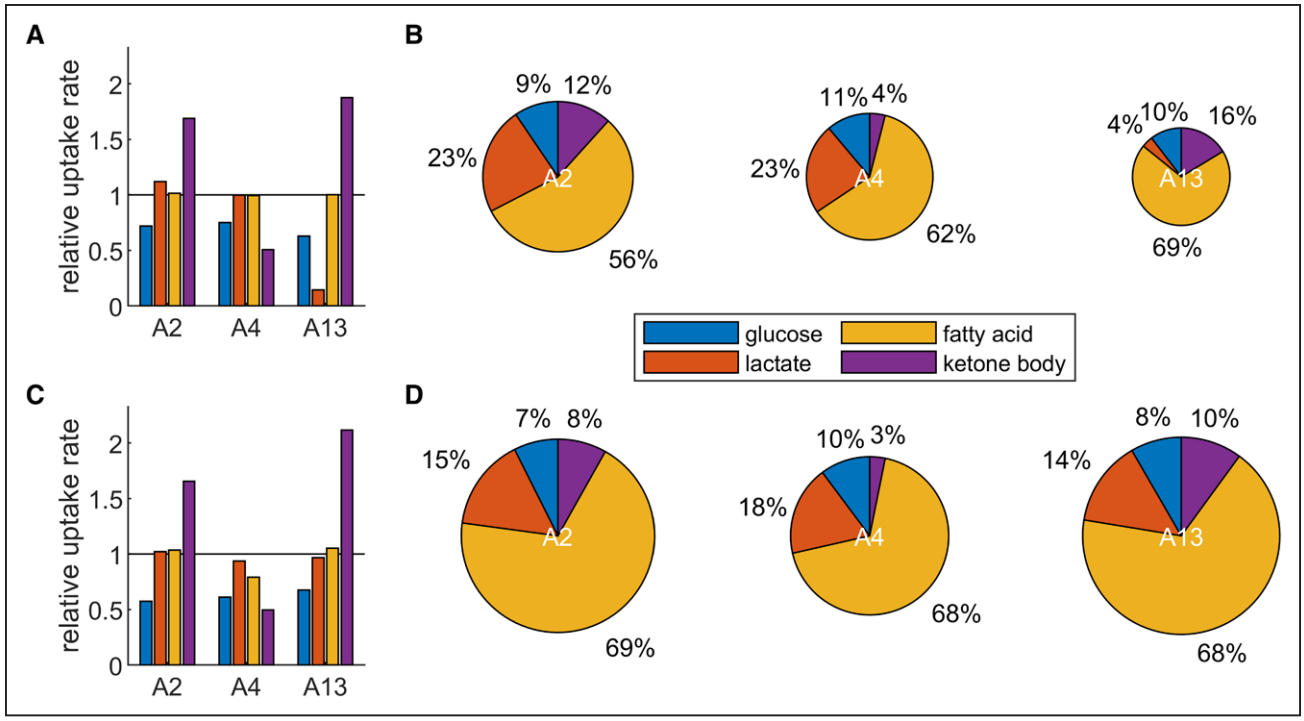

Figure 6. Metabolic characterization of 3 patients with aortic stenosis.
On the left, relative substrate utilization rates are shown compared with healthy controls at rest (**A**) and at maximal load (**C**). On the right, relative contribution of the different substrates (glucose [blue], lactate [orange], fatty acids [yellow], and ketone bodies [purple]) to overall ATP production rate at rest (**B**) and maximal load (**D**) are presented. Areas of pie diagrams represent total ATP production rate.

of restrictions imposed to heart performance by the nonmetabolic factors. To close this methodologic gap, we applied a novel approach to assess to energetic capacity of the LV of the human heart by combining kinetic modeling with protein abundance data of metabolic enzymes determined in cardiac tissue.

Reduced Myocardial Energetics in Patients With Valvular Disease

AS and MVI lead to chronic pressure or volume overload that result in phenotypically different myocardial remodeling (eccentric and concentric hypertrophy, respectively), both of which can progress to heart failure if left untreated. The cardiac adaptation processes to chronic overload conditions have been well characterized in terms of phenotyping and analysis of global cardiac function, but little is known about metabolic changes. The central findings of our approach are that even in patients with valvular dysfunction but preserved systolic function and no sign of heart failure, the energy metabolism is already deteriorated (Figure 3) and closely associated with mechanical power and systolic performance (Figure 5). The first finding is in line with several studies (reviewed in Sankaralingam and Lopaschuk),[49] which have established that a reduction in the ATP production capacity already occurs in early phases of heart failure development. The second finding identifies the capability of the cardiac metabolic network to generate ATP as the key link between systolic function and energy metabolism of the LV rather than the intracellular transport capacity of energy-rich phosphates by the creatine kinase shuttle, which was found to not be significantly different in patients with AS with preserved and reduced systolic function.[10]

MV_{ATP}(rest) was significantly increased in the MVI and AS groups and MV_{ATP}(max) was significantly decreased in both groups, resulting in a significant reduction of the specific ATP production reserve MAPR (Figure 3). The general decrease of MV_{ATP}(max) in both groups of patients can be accounted for by a decrease of the oxidative phosphorylation capacity as none of the investigated LVs showed excessive glycolytic activity. A decreased expression of the PGC-1α/PPARα transcription cascade has been identified as an important mechanism responsible for downregulation of the oxidative phosphorylation in the failing myocardium.[3]

Our analysis revealed in both groups of patients a large variability of the energy parameters (Figures 3 and 6), likely reflecting larger differences in the patient-specific functional and structural response of the LV to pressure/volume overload. Whereas some patients present with signs of hypertrophy, myocardial thickening, and ventricular dilation, others may show alterations in contraction time or only modest signs of remodeling.[50,51] It is in the nature of our approach that the large interindividual variabilities in the computed metabolic parameters are exclusively attributable to individual variabilities in the abundance of metabolic enzymes and transporters, and

these may be caused by individual differences in transcription, translation, or proteolysis. In a recent study, it was found that LV with normal aortic valves and with AS and aortic insufficiency disease all exhibit unique transcriptional profiles.[52] Thus, it appears more likely that the observed metabolic heterogeneity is caused by small RNA variabilities, including microRNAs and small transfer RNA fragments that can operate as modulators of the altered proteomics state.[53,54]

The large intraindividual variability of cardiac energetics in patients with valvular dysfunction necessitates an individual assessment of the metabolic status (Figure 6).

Our study revealed a significant correlation between improvement in NYHA staging after valve surgery and a substantial number of preoperative metabolic capacities. In line with this, Pasquet et al[55] identified cardiac glycolytic activity, assessed by means of 18-fluorodeoxyglucose positron emission tomography, as predictor of postoperative myocardial recovery after bypass surgery in patients with severe LV dysfunction. In principle, our approach allows inclusion of a large panel of metabolic capacities in the definition of novel risk predictors of postoperative outcome, but the additional benefit of such metabolic markers remains to be examined.

Alterations in the Myocardial Substrate Preference in Patients With Valvular Disease

Both groups of patients exhibited significant changes in myocardial use of the main energy substrates. There was a trend toward higher uptake rates for glucose and decreased uptake rates for lactate in patients with MVI and a decrease in glucose as well as lactate use in patients with AS. KB use rates showed high variability, but were generally increased in in patients with AS. This is in line with recent studies suggesting that increased reliance on KB metabolism offers a metabolic advantage in the failing heart and an ergogenic aid for exercise performance.[56,57]

Limitations of Our Approach

Our approach is restricted to the assessment of the energetic performance of an average cardiomyocyte working at fixed external concentrations of substrates and hormones. Hence, individual variances in circulating nutrients and hormones as well as activity-dependent concentration changes of oxygen and substrates attributable to changes of the coronary blood flow and the systemic metabolism (in particular skeletal muscle) were not taken into account. Moreover, it is well established that myocardial blood flow is heterogeneous on the local level and that this heterogeneity in perfusion entails heterogeneity in the metabolic endowment of cardiomyocytes.[58] For example, Bach et al.[59] demonstrated an association between heterogeneity of ventricular function and myocardial oxidative metabolism in nonischemic dilated cardiomyopathy. Thus, our analysis provides a comparison of metabolic capability under standardized conditions. The next step will be to incorporate the cellular metabolic model into a tissue-scale model of myocardial metabolism that includes local heterogeneity of blood flow, protein abundance, as well as individual plasma nutrient and hormone concentrations. Our previous work on liver metabolism[19,60,61] may serve as a paradigm for such a stepwise advancement of metabolic models from the cellular to the tissue level, but more in-depth data, such as histologic characterization and plasma metabolomics, will be required to enable such an approach.

Regarding the usefulness of our approach for the clinical assessment of heart diseases, the need for protein abundance data is the decisive limiting factor, because endomyocardial biopsies are most commonly used in surveillance of allograft rejection in patients who undergo heart transplant.

Conclusions

We investigated 2 different cohorts (AS and MVI) with large biophysical differences in terms of ventricular remodeling but comparable clinical state and ventricular function. Our approach can unravel differences in the energetic state of the myocardium even in hearts that have similar clinical markers of hypertrophy and pump function. The proposed model-based approach extends our capabilities to gain deeper insight into metabolic alterations in different types of heart diseases.

ARTICLE INFORMATION

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Disclosures

Patent application EP21174633, "Computer-assisted method for the evaluation of cardiac metabolism," was filed by Charité-Universitätsmedizin Berlin as employer of Dr Berndt and Prof Kuehne, with both holding inventorship for this patent application. The other authors report no conflicts.

Supplemental Material

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