J Mol Med (2009) 87:411–422
DOI 10.1007/s00109-008-0436-x

ORIGINAL ARTICLE

Decreased contractility due to energy deprivation in a transgenic rat model of hypertrophic cardiomyopathy

Mark Luedde · Ulrich Flögel · Maike Knorr · Christina Grundt · Hans-Joerg Hippe · Benedikt Brors · Derk Frank · Uta Haselmann · Claude Antony · Mirko Voelkers · Juergen Schrader · Patrick Most · Bjoern Lemmer · Hugo A. Katus · Norbert Frey

Received: 17 July 2008 / Revised: 18 November 2008 / Accepted: 10 December 2008 / Published online: 3 February 2009

Abstract Hypertrophic cardiomyopathy (HCM) is associated with cardiac hypertrophy, diastolic dysfunction, and sudden death. Recently, it has been suggested that inefficient energy utilization could be a common molecular pathway of HCM-related mutations. We have previously generated transgenic Sprague–Dawley rats overexpressing a truncated cardiac troponin T (DEL-TNT) molecule, displaying typical features of HCM such as diastolic dysfunction and an increased susceptibility to ventricular arrhythmias. We now studied these rats using 31P magnetic resonance spectroscopy (MRS). MRS demonstrated that cardiac energy metabolism was markedly impaired, as indicated by a decreased phosphocreatine to ATP ratio (−31%, $p<0.05$). In addition, we assessed contractility of isolated cardiomyocytes. While DEL-TNT and control cardiomyocytes showed no difference under baseline conditions, DEL-TNT cardiomyocytes selectively exhibited a decrease in fractional shortening by 28% after 1 h in glucose-deprived medium ($p<0.05$). Moreover, significant decreases in contraction velocity and relaxation velocity were observed. To identify the underlying molecular pathways, we performed transcriptional profiling using real-time PCR. DEL-TNT hearts exhibited induction of several genes critical for cardiac energy supply, including CD36, CPT-1/-2, and PGC-1α. Finally, DEL-TNT rats and controls were studied by radiotelemetry after being stressed by isoproterenol, revealing a significantly increased frequency of arrhythmias in transgenic animals. In summary, we demonstrate profound energetic alterations in DEL-TNT hearts, supporting the notion that inefficient cellular ATP utilization contributes to the pathogenesis of HCM.

Keywords Cardiomyopathy · Transgenic · Troponin T · Energy metabolism

Electronic supplementary material The online version of this article (doi:10.1007/s00109-008-0436-x) contains supplementary material, which is available to authorized users.

M. Luedde · M. Knorr · H.-J. Hippe · D. Frank · M. Voelkers · H. A. Katus
Department of Cardiology,
University of Heidelberg,
Heidelberg, Germany

N. Frey (✉)
Department of Cardiology and Angiology,
University of Kiel,
Schittenhelmstraße 12,
24105 Kiel, Germany
e-mail: freynorbert@hotmail.com

U. Flögel · J. Schrader
Department of Cardiovascular Physiology,
Heinrich-Heine-University,
Mannheim, Germany

C. Grundt · B. Lemmer
Institute of Experimental & Clinical Pharmacology & Toxicology,
Medical Faculty Mannheim, University of Heidelberg,
Mannheim, Germany

B. Brors
Department of Theoretical Bioinformatics,
German Cancer Research Center,
Heidelberg, Germany

U. Haselmann · C. Antony
European Molecular Biology Laboratory,
Heidelberg, Germany

P. Most
Center for Translational Medicine, Department of Medicine,
Thomas Jefferson University,
Philadelphia, USA

Introduction

Hypertrophic cardiomyopathy (HCM) is an inherited heart disease, affecting approximately 0.2% of the population [1]. The clinical phenotype is highly variable, ranging from lifelong absence of symptoms to progressive heart failure or early sudden cardiac death in adolescents. Multiple mutations in more than ten genes have been shown to cause HCM, most of which encode for contractile proteins [2]. While the perception of HCM as a disease of the sarcomere [3] is thus widely accepted, the pathogenetic mechanisms by which HCM-associated mutations cause the disease still remain unclear. Exemplarily, the alterations of contractility caused by different sarcomeric protein mutations are not consistent. Mutations in the β-myosin heavy chain may result in either reduced [4] or enhanced motor activity [5].

Next to β-myosin heavy chain and myosin binding protein C, mutations in the cardiac troponin T (TNT) gene are a frequent cause of HCM and more than 30 HCM-associated TNT mutations have been described. Clinically, TNT mutations are characterized by relatively mild or even absent cardiac hypertrophy but nevertheless a high incidence of ventricular arrhythmias and sudden death [6]. TNT forms the troponin complex together with troponin C and I and binds to tropomyosin, thereby modulating thin filament function by several mechanisms, including regulation of Ca^{2+} sensitivity, the conformational state of the thin filament, and subsequently the magnitude of myofibrillar ATPase activity [7] as well as the level of force generation [8].

A recent theory considers "energy compromise" due to inefficient ATP utilization of the sarcomere as a central defect in HCM [9]. This view is supported by the observation that several different mutations produce an impaired phosphocreatine (PCr)/ATP ratio in affected patients [10]. Moreover, defects in genes critical for myocardial energy supply, such as CD36 [11] and mitochondrial tRNA [12], cause a cardiac phenotype that closely resembles HCM.

We have previously generated a transgenic rat model of the disease, expressing a human TNT truncation mutation lacking exon 16 (DEL-TNT). DEL-TNT transgenic hearts display an impaired systolic and diastolic function in the working heart model in the absence of significant left ventricular hypertrophy [13]. Moreover, after exercise training, these hearts exhibited ventricular arrhythmias, corresponding to the high risk of patients with this mutation for sudden cardiac death [13]. Finally, investigations on skinned DEL-TNT cardiac fiber preparations revealed an impaired ratio of calcium-dependent tension development and myofibrillar ATPase activity [14].

We now assessed whether energy homeostasis is compromised in DEL-TNT rats in vivo. In addition, contractile function of energy-depleted DEL-TNT cardiomyocytes and WT controls was examined in vitro.

Here, we demonstrate profound energetic alterations in DEL-TNT hearts, which lead to contractile dysfunction under conditions of metabolic stress. These findings support the theory that a cellular energy deficit is an important pathogenetic factor that may contribute to the clinical phenotype of HCM.

Materials and methods

Please see the online supplement for details on procedures for magnetic resonance studies, isolation and culture of adult rat ventricular cardiomyocytes, measurement of contractile parameters, transmission electron microscopy, RNA isolation, quantitative real-time PCR analysis of gene expression, and recording of cardiovascular parameters in transgenic rats.

Animals

Transgenic Sprague–Dawley rats expressing a human cTnT deletion mutation (DEL-TNT) were generated as described [13]. The investigation conforms to 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). All experimental procedures in this study have been performed using rats at the age of 4 to 6 months except for MRI studies that have been performed with 4-week-old rats.

Magnetic resonance studies

To obtain an integrated analysis of cardiac function and energetics in vivo, a combined approach of MRI and 31P MRS was applied, as previously described [15]. For quantification of myocardial PCr/ATP ratios, only voxels covering the free left ventricular wall were considered, since in both groups spectra of septal voxels were occasionally contaminated with 31P signals originating from chamber blood as reflected by the appearance of 2,3-diphosphoglycerate (DPG) signals (data not shown).

Measurement of contractile parameters of adult rat ventricular cardiomyocytes

Adult rat cardiomyocytes (ARCM) were isolated from Sprague–Dawley rats. Twenty four hours after plating, cells were incubated for 1 h in either Dulbecco's Modified Eagle Medium (DMEM) containing glucose (100 mg/dl) or in glucose-depleted DMEM containing 1 mM 2-deoxy-D-glucose (2-DOG) at 37°C. Subsequently, single-myocyte contractility was analyzed at 25°C, using a video edge detection system [23] (Fig. 2). A total of $n=65$ CMs (derived from eight DEL-TNT hearts) and 60 control cardiomyocytes

(of eight wild-type littermates) were examined by an investigator blinded to the genotype. Contractile parameters in ARCM were obtained by video edge detection.

Statistical analyses

All results are shown as the mean ± SEM. Real-time PCR data analyses were carried out using the ΔΔct method [16]. Statistical significance was determined using Student's unpaired *t* test or Fisher's exact test for the analysis of categorical data. Values of $p<0.05$ were considered statistically significant.

Results

^{31}P magnetic resonance spectroscopy reveals altered cardiac energy metabolism of DEL-TNT transgenic rats in vivo

To investigate cardiac function of DEL-TNT rats in vivo and to correlate these data to potentially altered energetics, we utilized an integrated MRI/^{31}P MRS approach in 4-week-old DEL-TNT and WT rats as described [15]. Basic functional cardiac parameters of WT and DEL-TNT rats are summarized in Table 1. In line with our previous data in 10- to 12- and 30- to 38-week-old DEL-TNT rats [13] and other transgenic animal models of cTNT-related HCM [17], transgenic hearts displayed no cardiac hypertrophy, as reflected by an identical heart weight/body weight ratio. Furthermore, ejection fraction as well as ventricular volumes and diameters were comparable in DEL-TNT animals and wild-type controls. In contrast, 2D ^{31}P chemical shift imaging (CSI) revealed a marked decrease of cardiac PCr levels in DEL-TNT hearts. Figure 1 shows representative ^{31}P MR spectra extracted from full CSI data sets of WT and transgenic rats in correlation with the anatomical ^{1}H MR image. Table 2 displays PCr/ATP ratios specified for individual wall regions of the left ventricle. Averaging the high energy phosphate levels over posterior, lateral, and anterior walls (Fig. 1b) revealed a significantly decreased phosphocreatine-to-ATP ratio (PCr/ATP) (−31%; $p<0.05$; Fig. 1) of the entire left ventricle. The observed effects were caused by a drop in PCr levels since no differences in ATP levels between the groups were detected and total creatine pools were similar for WT and mutants (Fig. 1, Table 3). These data suggest that cardiac energy turnover is markedly increased in DEL-TNT transgenic rat hearts.

Table 1 Basal cardiac parameters of WT and DEL-TNT rats as assembled by MRI in vivo

Parameter	WT	DEL-TNT
Animal weight (g)	64.80±2.79	66.66±4.28
Heart weight (mg)	251.70±16.29	244.26±20.21
Heart body index (mg/g)	3.87±0.14	3.64±0.11
End-diastolic volume (μl)	142.62±8.62	126.73±4.62
End-systolic volume (μl)	47.98±4.45	38.75±4.41
Stroke volume (μl)	94.63±5.05	87.98±3.88
Ejection fraction (%)	66.71±1.59	69.65±2.90
Heart rate (bpm)	393.87±7.33	397.52±13.35
Cardiac output (ml/min)	37.12±1.72	35.03±2.11
End-diastolic wall diameter (mm)	1.21±0.03	1.21±0.07
End-systolic wall diameter (mm)	1.86±0.04	1.89±0.09
Systolic wall thickening (%)	54.24±1.57	58.52±8.45

The values are means ± SEM (WT $n=7$; DEL-TNT $n=6$). All comparisons p = n.s.

Metabolic stress differentially impairs contractile function of DEL-TNT transgenic cardiomyocytes

To further investigate the pathophysiological relevance of the observed alterations in cardiac energy metabolism, we examined the contractile function of DEL-TNT transgenic adult (4–6 months old) rat ventricular cardiomyocytes (CMs) in comparison to wild-type (WT) controls. The presence of glucose-containing DMEM, fractional cell shortening was not significantly different in DEL-TNT CMs compared to WT controls (19.9%±0.8% vs. 18.8%±0.6%; Fig. 2a, b). However, after 1 h of glucose depletion, there was still no significant decline in fractional shortening of WT controls (−10%±4.9%), while DEL-TNT cardiomyocytes revealed a significant reduction (−28%±6.0%, $p<0.05$; Fig. 2a, b). Measures of systolic and diastolic contraction velocity were also differentially impaired in DEL-TNT CMs, including myocyte shortening (−dl/dt=−32.3% (±7.4%) in DEL-TNT vs. −0.06% (±7.7%) in WT, $p<0.01$; Fig. 2c) and relengthening (+dl/dt=−26.8% (±6.1%) in DEL-TNT vs. +1.0% (±8.05%) in WT, $p<0.05$; Fig. 2d). To investigate if increased energy demand would exacerbate the decline in fractional shortening of DEL-TNT cardiomyocytes, we additionally incubated the cells in 10 nm isoproterenol (ISO)-containing medium 5 min before measurement of contraction (Electronic supplementary material). WT and DEL-TNT CMs responded with a highly significant increase in fractional shortening after ISO stimulation (WT=+108% [±7.15%]; DEL-TNT=+87% [±13.9%]; $p<0.001$). Yet, after 1 h of glucose depletion, again only DEL-TNT CMs revealed a reduction in fractional shortening (−46%± 9.83%; $p<0.05$). Likewise, in ISO-stressed DEL-TNT cells, a marked and highly significant reduction of fractional shortening upon metabolic stress was observed (−47%± 6.05%; $p<0.001$). Thus, we observed that, although basal contractility was not altered in DEL-TNT cardiomyocytes, these cells displayed an exaggerated susceptibility for contractile dysfunction under conditions of both decreased energy supply and increased energy demand compared to WT controls.

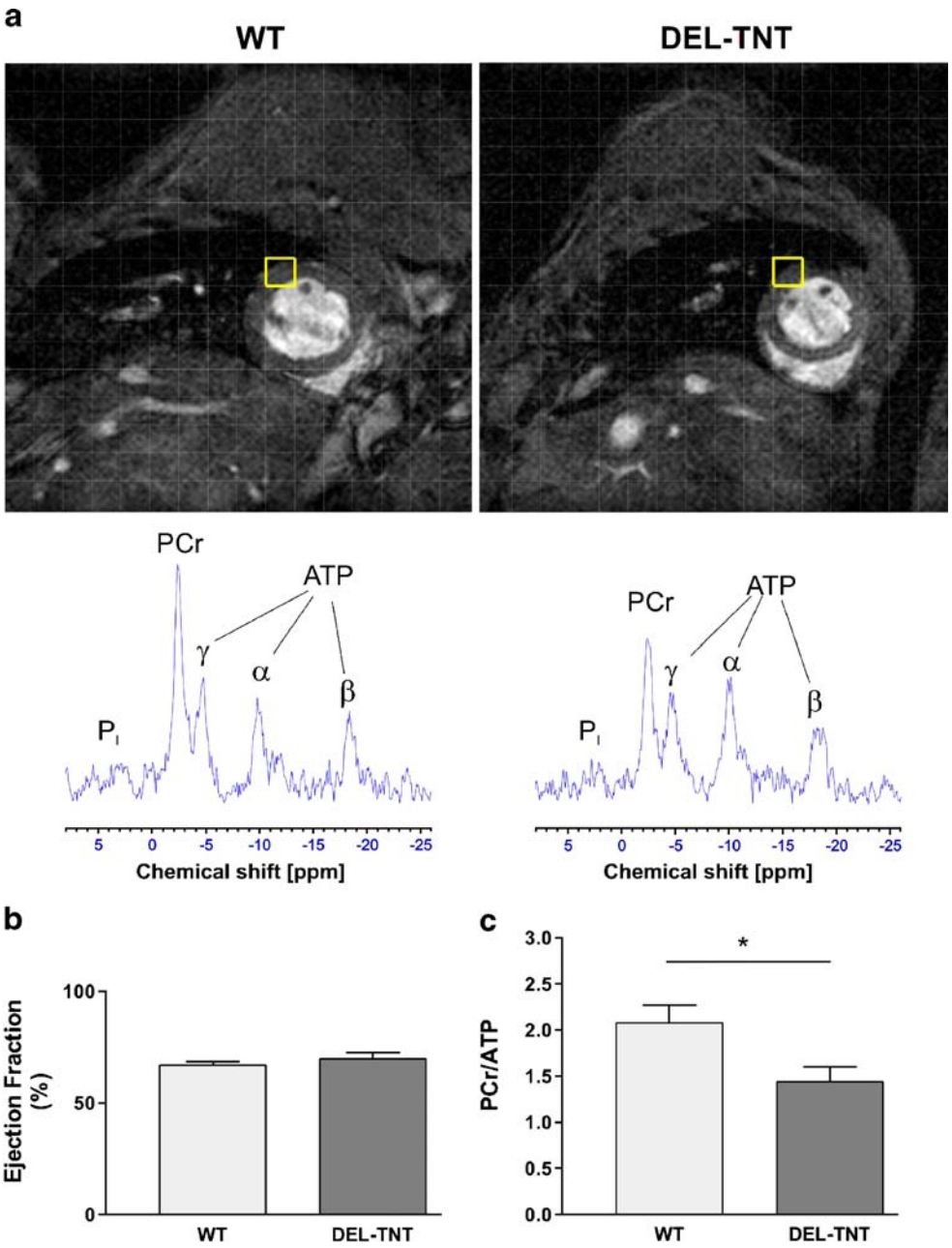

Fig. 1 Decreased phosphocreatine-to-ATP ratio of DEL-TNT rat hearts in vivo. **a** End-diastolic short axis ^{1}H MR images from a field of view of 30×30 mm^2 and corresponding representative ^{31}P MR spectra from selected voxels of the anterior LV wall. Characteristic spectra show that PCr/ATP ratio is lower in the transgenic rat compared to the WT control. **b** Ejection fraction of all DEL-TNT transgenic rats (n=6) and controls (n=7). **c** Left ventricular phosphocreatine/adenosine triphosphate (PCr/ATP) ratios averaged over posterior, lateral, and anterior wall. Data are mean ± SEM. *p<0.05

Induction of mitochondrial biogenesis in DEL-TNT rat hearts

It is conceivable that a latent energy deficit may lead to an increased mitochondrial biogenesis in DEL-TNT cardiomyocytes. Thus, the fractional cell area occupied by mitochondria was assessed in adult (6 months old) transgenic rat hearts compared to WT littermates using transmission electron microscopy (TEM). Fractional mitochondrial area reflects both the number and size of mitochondria. Figure 3a shows representative TEM images of ventricular sections from WT (left) and DEL-TNT rats (right). Measurement of n=45 transgenic cardiomyocyte sections compared to n=43 WT controls revealed a significant increase in the mitochondrial fraction (+17.9%, p<0.01) in DEL-TNT ventricular sections (Fig. 3b). These data suggest that an extended mitochondrial fraction may serve to compensate for a chronically increased energy demand in DEL-TNT transgenic rat hearts.

Differential expression of metabolic genes in DEL-TNT rat hearts

To investigate the molecular basis of the metabolic phenotype of DEL-TNT rats, we analyzed cardiac gene expression levels at the age of 6 months by quantitative

Table 2 PCr/ATP ratios specified for individual left ventricular walls of WT (n=5) and DEL-TNT (n=6) rats

	WT	DEL-TNT
Lateral left ventricular wall	2.10±0.23	1.38±0.16*
Anterior left ventricular wall	2.07±0.21	1.45±0.18*
Posterior left ventricular wall	2.07±0.25	1.49±0.16*

*p<0.05

real-time PCR. We chose to analyze a broad range of genes involved in various aspects of cardiac energy metabolism, including molecules important for energy supply and fatty acid metabolism as well as critical regulators of the cardiomyocyte's metabolic state. The target genes were normalized using oligonucleotide primers for β-actin as an internal standard. β-Actin was not differentially regulated in DEL-TNT rats vs. WT controls (data not shown). The PCR results of several genes were also assessed on the protein level by Western blot analysis.

Upon real-time PCR analysis, several enzymes involved in energy generation were found to be upregulated in DEL-TNT rat hearts, including the mitochondrial Fo-complex of the ATP synthase (+78%, p<0.01), which is directly involved in ATP synthesis in the mitochondria as well as soluble malate dehydrogenase, which catalyzes the final step of the citric acid cycle (+28%, p<0.05; Fig. 4).

Expression of fatty acid oxidation genes is controlled by the PPAR-γ coactivator 1α (PGC-1α) [18]. PGC-1α, which also acts as a key regulator of mitochondrial biogenesis [19], was found upregulated in DEL-TNT hearts on the mRNA level (+170%, p<0.05) and on the protein level (+16.8%, p<0.05%; Fig. 4a, c). Furthermore, malonyl-CoA decarboxylase, another major regulator of mitochondrial fatty acid uptake and oxidation, was significantly induced in DEL-TNT rats (+94%, p<0.001). Fatty acid transporter FAT/CD36 and the carnitine palmitoyltransferases M-CPT-1 and CPT-2 represent critical steps in the fatty acid oxidation pathway. Transcripts encoding these molecules were significantly upregulated in DEL-TNT hearts (CD36=+138%, p<0.01; CPT-1=+55%, p<0.01; CPT-2=+46%, p<0.05). Western blot analysis revealed an upregulation of CD 36 of +24% (p<0.05, Fig. 4c).

Taken together, these findings demonstrate an upregulation of major pathways of cardiac energy production in DEL-TNT rat hearts, which might represent a compensatory mechanism for the observed impaired cardiac energetics.

Table 3 Quantification of ATP, creatine, and PCr concentrations of WT (n=5) and DEL-TNT (n=6) rats

Parameter	WT	DEL-TNT
ATP (mM)	6.45±1.17	6.27±1.44
Creatinine (mM)	18.63±1.57	19.36±1.89
PCr (mM)	13.41±3.35	9.01±2.60*

*p<0.05

Increased susceptibility for ventricular arrhythmias in DEL-TNT transgenic rats

Isoproterenol has been demonstrated to increase myocardial energy turnover and to decrease the economy of contraction in failing and non-failing myocardium [20]. Moreover, we have previously observed that isolated DEL-TNT transgenic rat hearts display ventricular arrhythmias. To now assess the consequences of acute isoproterenol infusion in vivo, we monitored heart rate and rhythm of 4- to 6-month-old DEL-TNT rats (n=7) and WT controls (n=5) under basal resting conditions and after injection of isoproterenol at increasing concentrations.

The injection of increasing dosages of isoproterenol had no differential effect on heart rate of DEL-TNT and WT rats, respectively (Fig. 5a, b). In contrast, DEL-TNT rats displayed significantly more premature ventricular complexes than WT rats, both under basal conditions and following the injection of isoproterenol (Fig. 5c, d). Moreover, we also observed a marked increase in the frequency of ventricular triplets in DEL-TNT rats vs. WT, in particular after injection of the highest dose of isoproterenol (64 μg/kg) (Fig. 5e, f). Finally, five out of seven DEL-TNT rats and no WT control displayed ventricular tachycardias (VTs) after injection of isoproterenol (p<0.05, data not shown), and one DEL-TNT rat died from sudden cardiac death due to ventricular fibrillation 12 min after injection of 64 μg/kg isoproterenol (Fig. 5g).

In conclusion, these findings demonstrate an increased susceptibility of DEL-TNT rats for ventricular arrhythmias after isoproterenol injection in vivo.

Discussion

Hypertrophic cardiomyopathy (HCM) is associated with cardiac hypertrophy, diastolic dysfunction, and an increased risk of sudden death. While HCM could be linked to multiple mutations in sarcomeric proteins [1], its pathogenesis is still poorly understood. Recently, inefficient energy utilization has been suggested as a common molecular pathway of HCM-associated mutations. In an attempt to unravel the underlying molecular events, we have previously generated transgenic rats overexpressing a truncated human cardiac troponin T molecule (DEL-TNT). In this model, contractile dysfunction as well as an enhanced susceptibility for ventricular arrhythmias was observed [13]. These findings were associated with a marked increase in myofibrillar ATP consumption and thus an inefficient energy utilization [14]. In the present study, we

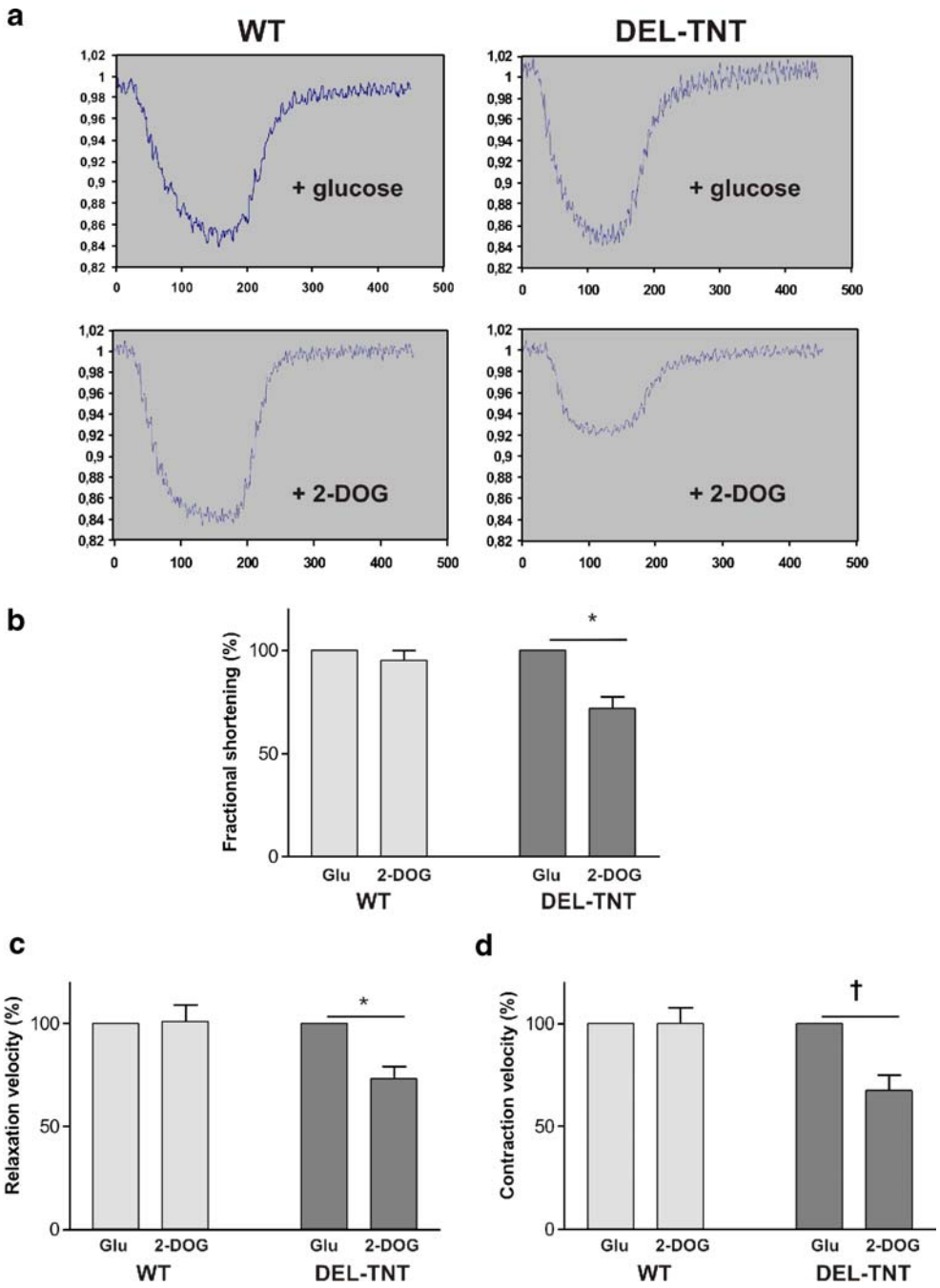

Fig. 2 Impaired contractile function of DEL-TNT cardiomyocytes due to metabolic stress. Contractility of isolated cardiomyocytes (CM) was analyzed by video edge detection. Sixty five CMs derived from eight DEL-TNT hearts and 60 CMs of eight WT controls were analyzed. **a** Representative tracings of contractions under different metabolic conditions are displayed, revealing no significant decline in contractility of WT or DEL-TNT cardiomyocytes after 1 h of glucose deprivation (*upper two boxes*). In contrast, there was a marked decrease of contractility of DEL-TNT CMs (*bottom boxes*) after 1 h of glucose depletion. **b** Quantification of contractile parameters revealed a decrease of fractional shortening (FS) of −28% in DEL-TNT CMs (±6.0%) due to glucose depletion as well as a decline of contraction velocity (**c**) (−32.34%± 7.4%) and relaxation velocity (**d**) (−26.82%±6.1%). These parameters were not significantly altered in WT controls. Data are mean values ± SEM. *$p<0.05$, †$p<0.01$, ‡$p<0.001$

now corroborate and extend these experiments using both an in vitro and an in vivo approach, supporting the notion that altered cardiomyocyte energy metabolism may play an important role in the pathogenesis of HCM.

Altered energetics in vivo

HCM has been labeled a "disease of the sarcomere" [3], as many different mutant alleles in at least nine genes encoding for contractile proteins have been found to give rise to the pathological phenotype. However, a unifying hypothesis of the pathogenesis has been hampered by the fact that different HCM-causing mutations have no consistent effects on the force-generating capacities and/or calcium sensitivity of the sarcomere. For example, only a minority of HCM-associated sarcomeric protein mutations result in reduced force generation in in vitro assays, which could potentially account for compensatory hypertrophy. On the contrary, several mutations rather *enhance* contractility [21].

Recently, inefficient energy utilization and consequently an increased energy demand of the sarcomere have been

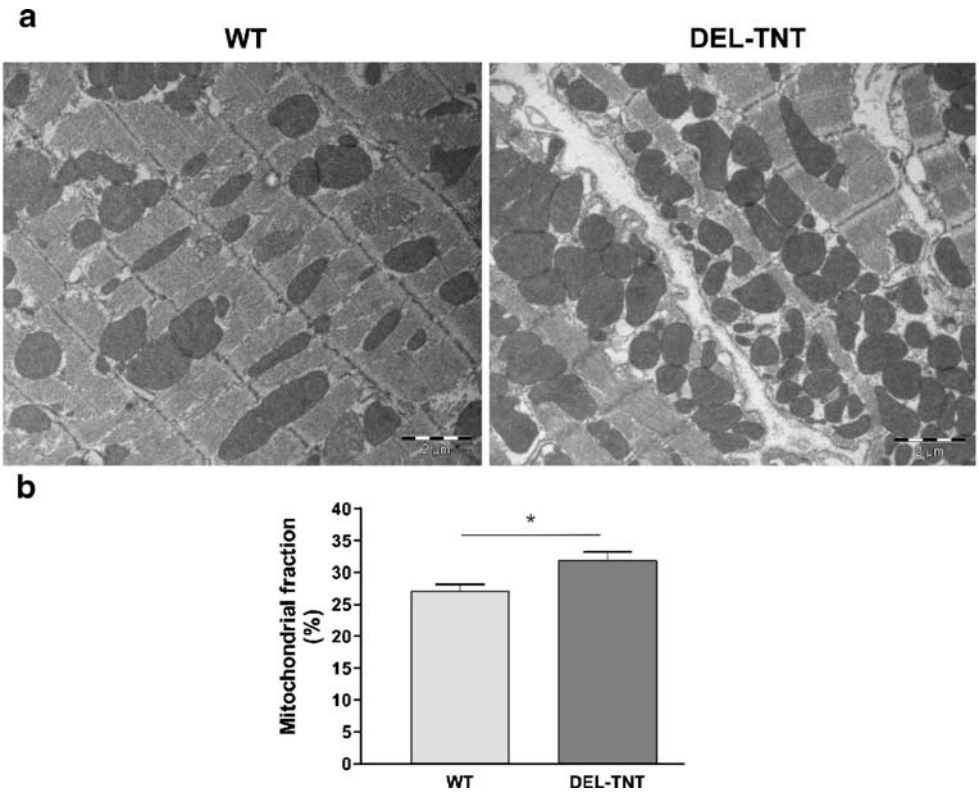

Fig. 3 Extended mitochondrial fraction in DEL-TNT transgenic rat hearts. **a** Electron microscopy of ventricular tissue sections. Images show the ultrastructure of ventricular tissue from wild-type (*left*) or DEL-TNT (*right*) animals. **b** Quantification of the mitochondrial fraction in 45 sections from three DEL-TNT rat hearts and 43 sections from three WT controls. *$p<0.01$

suggested as key features of many if not all HCM-associated mutations [22]. In terms of cross-bridge cycling efficiency, we and others could show that cardiac TnT mutations increase the cost of force production [14, 22]. According to this premise, an increased energy turnover rate of the sarcomere might compromise the ability of the cardiomyocyte to maintain sufficient energy levels for critical homeostatic functions, such as Ca^{2+} re-uptake [9], especially under conditions of increased demand such as exercise. Using 2D ^{31}P CSI, we demonstrate that cardiac energetics are in fact significantly impaired in DEL-TNT rats hearts in vivo, reflected by a decreased phosphocreatine to ATP ratio (PCr/ATP). Thus, energy wastage through inefficient chemo-mechanical transduction would lead to increased turnover of ATP in certain subcellular compartments, particularly during periods of stress. These data are in line with in vitro findings obtained in other models of HCM, e.g., αMHC403/+- [23] or (R92Q)-TnT [24]-transgenic mice. In these animal models, a decrease in PCr due to increased ATP utilization in explanted hearts could be demonstrated, resulting in an impaired ability of the heart to recruit its contractile reserve [24]. A decreased PCr/ATP ratio at the same order of magnitude (−30%) has also been observed in HCM patients harboring either cardiac troponin T mutations or mutations in other sarcomeric proteins [10]. It has been shown that even mutation carriers who did not display cardiac hypertrophy already revealed an abnormal PCr/ATP ratio, suggesting that energetic alterations are not secondary to hypertrophic growth but rather an early feature in the course of the disease. These findings are consistent with our observation that alterations of PCR/ATP ratio could already be detected in very young transgenic rats at the age of 4 weeks. Since DEL-TNT rat hearts do not reveal hypertrophy, neither at the age of 4 weeks (Table 1) nor at older age [13], yet reveal an increased myofibrillar energy cost of force production [14], "altered energetics" appears to be a primary and early feature of these hearts, rather than a secondary phenomenon.

Next, we aimed to establish whether the observed alterations are cardiomyocyte autonomous and therefore examined contractile function of isolated ventricular myocytes from adult DEL-TNT rats. These cells displayed normal contractility compared to WT cardiomycytes when incubated in glucose-containing medium. However, metabolic stress due to glucose depletion and addition of 2-DOG led to a significantly decreased shortening fraction as well as impaired relaxation parameters selectively in transgenic cardiomyocytes, whereas wild-type cells still revealed preserved contractility. In additional experiments, we also stimulated cardiomyocytes with isoproterenol to assess the consequences of an increased energy demand. After isoproterenol stimulation and glucose depletion, again only DEL-TNT cardiomyocytes revealed a decreased shortening fraction. Thus, while an increased ATP demand in DEL-TNT mutant hearts might still be compensated for under basal conditions, it may become limiting in situations of

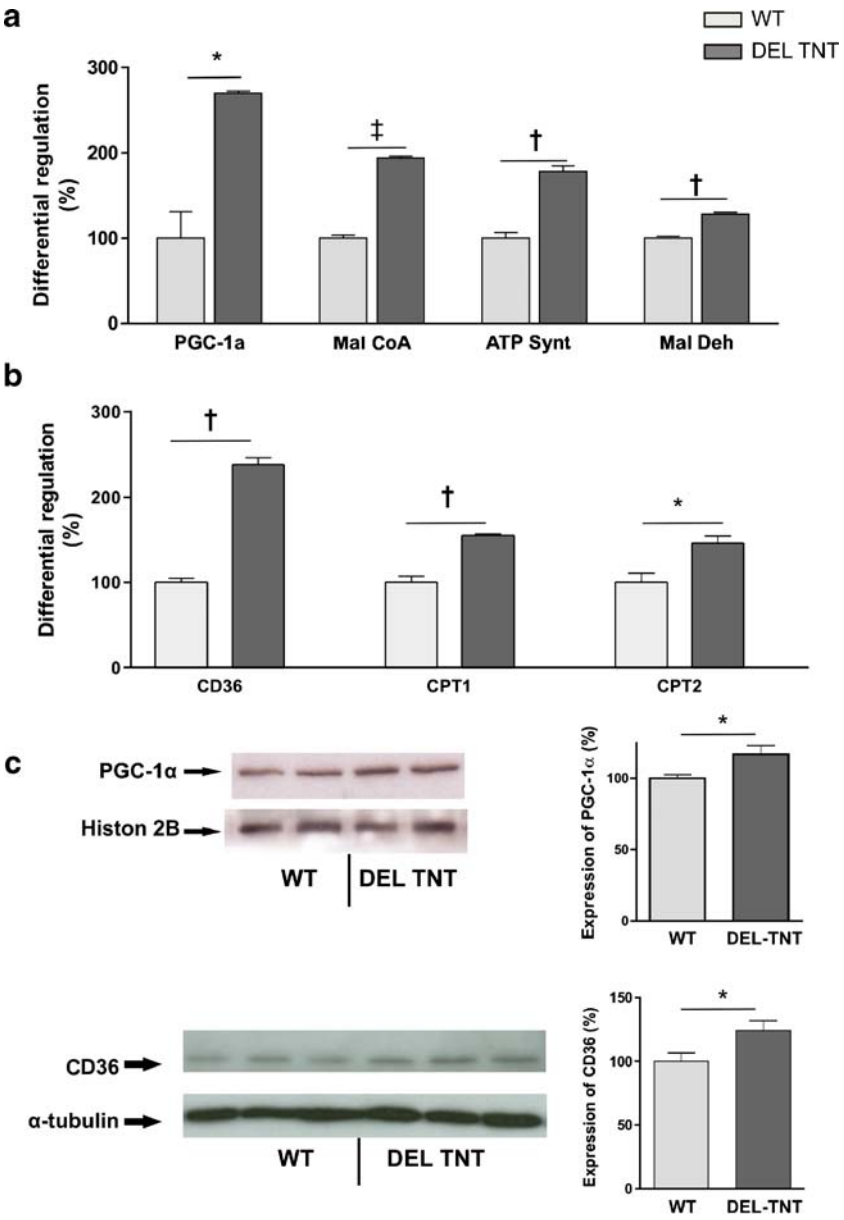

Fig. 4 Differential expression of metabolic genes in DEL-TNT transgenic rats. Real-time PCR data of metabolic genes that were found differentially regulated in DEL-TNT rat hearts (n=5) vs. WT controls (n=6). **a** Differential regulation of metabolic genes that control energy supply and mitochondrial biogenesis. **b** Upregulation of fatty acid transporter CD36 and the carnitine palmitoyltransferases M-CPT-1 and CPT-2 representing critical steps in the fatty acid oxidation pathway. **c** Representative Western blots and statistical analysis (n=4) revealing upregulation of PGC-1α by 16.8%±6.0% (nuclear extracts, loading control = histone 2B) and upregulation of CD 36 by 24.3%±7.8% (whole cell extracts, loading control = tubulin-α). Data are mean values± SEM. *p<0.05, †p<0.01, ‡p<0.001

increased cardiac work load, i.e., due to physical exertion. Conceivably, this could result in contractile dysfunction in vivo. Interestingly, a recent report revealed that HCM patients with troponin mutations display exercise-induced LV systolic dysfunction more frequently than HCM patients with mutations in other genes [25], pointing to a particular susceptibility of these patients to situations of increased energy demand.

Induction of mitochondrial biogenesis and differential regulation of metabolic genes

The theory of impaired energy transfer being central in the pathogenesis of the HCM phenotype is also supported by the fact that several inherited syndromes in which mitochondrial energy production is defective may result in asymmetric cardiac hypertrophy, clinically indistinguishable from HCM. These include abnormalities of mitochondrial function caused by mitochondrial tRNA mutations [12]. In DEL-TNT cardiomyocytes, we found a significant increase of the mitochondrial fraction by transmission electron microscopy. Mitochondrial proliferation has also been observed in histological studies of patients with hypertrophic cardiomyopathy due to other TNT mutations [26]. Together with data from a previous study that demonstrated an increased tension-dependent ATP consumption of DEL-TNT heart muscle fibers [14], these alterations could be interpreted as a compensatory response to the cellular energy deficit caused by inefficient sarcomeric energy utilization. Yet, the causative mechanism of

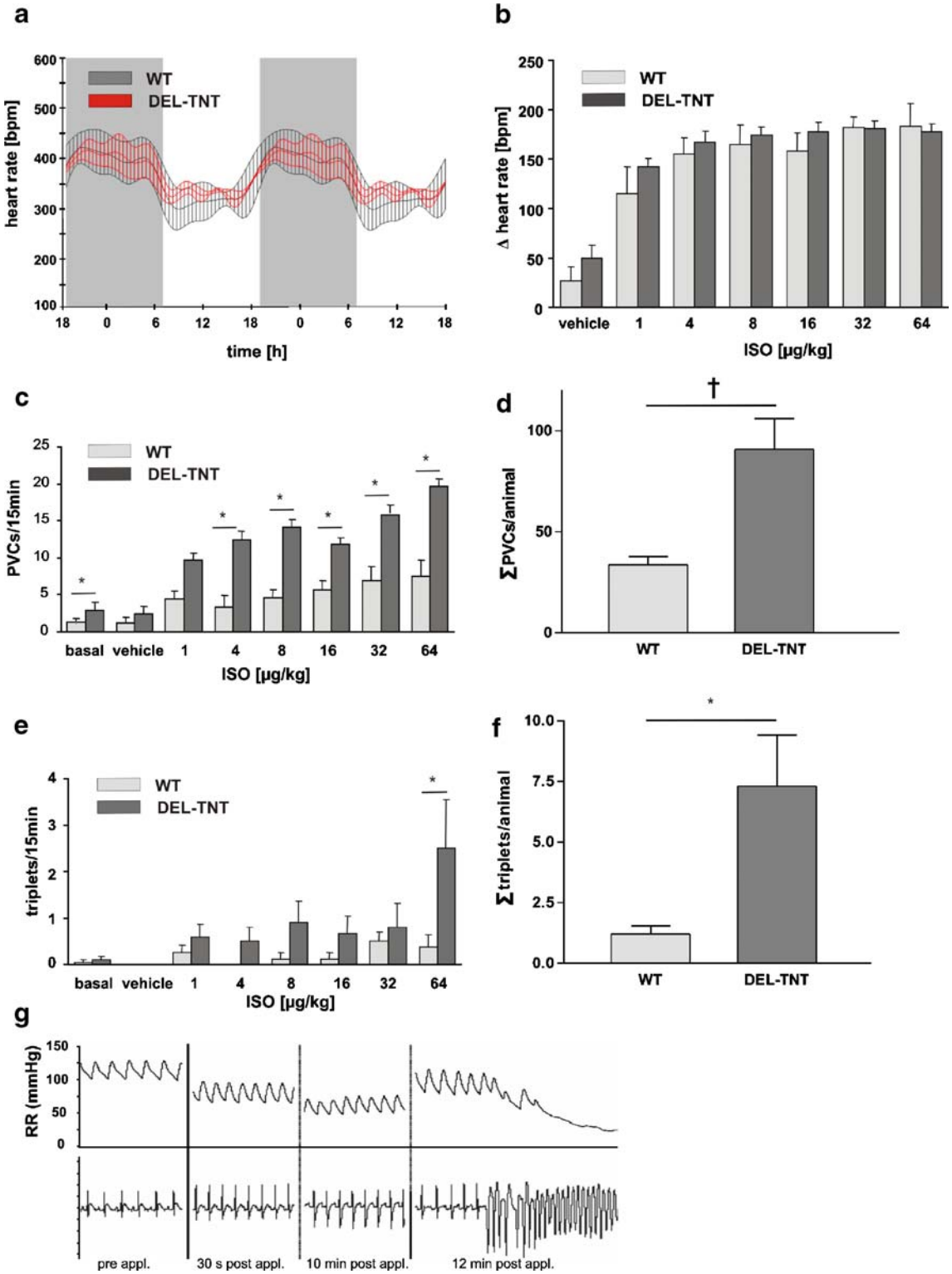

Fig. 5 Increased susceptibility of DEL-TNT rats for ventricular arrhythmias after isoproterenol injection in vivo. **a** Shown are the group mean values ± SEM of the circadian pattern of heart rate in WT (*gray*) (n=5) vs. DEL-TNT animals (*red*) (n=7). Rat's activity phases during darkness are underlayed in *gray color*. Telemetric ECG monitoring reveals a preserved 24-h heart rate variability in transgenic animals. **b** Increase in heart rate after injection of ascending dosages of isoproterenol (*ISO*) in both transgenic rats and controls. **c** Increased frequency of premature ventricular complexes (*PVCs*) under ascending dosages of ISO in DEL-TNT animals. **d** Sum of all PVCs/animal under basal conditions and ascending dosages of ISO. **e** Registration of triplets under basal conditions and after injection of increasing concentrations of ISO, again revealing a significant increase in transgenic rats. **f** Sum of all triplets/animal. **g** Blood pressure (*RR*) and ECG recording of a DEL-TNT rat that died of cardiac arrest due to ventricular fibrillation after application of 64 µg/kg ISO. Data are mean values ± SEM. *p< 0.05; †p<0.001

increased contractile protein energy turnover triggered by this troponin T mutation still needs to be exactly delineated. Given that the C-terminus of TNT binds to troponin C and I, it seems likely that mutations in this region influence myofibrillar cooperativity, which in turn may affect the conformation of actin, ultimately resulting in energy-inefficient cross-bridge cycling [14].

Of note, inherited defects in fatty acid uptake due to CD36 deficiency [11] also lead to a phenotype closely resembling HCM. In our experimental model, we found CD36 as well as the carnitine palmitoyltransferases CPT-1 and CPT-2 to be induced, the latter being responsible for mitochondrial import of fatty acids and controlling the rate-limiting steps in the mitochondrial fatty acid oxidation pathway [27]. These genes are co-activated by PGC-1α, an important regulating protein of mitochondrial biogenesis [18] which was also found to be upregulated in DEL-TNT hearts. Moreover, ATP synthase and malonyl-CoA decarboxylase, which trigger fatty acid oxidation by degradation of cardiac malonyl-CoA, were significantly induced in transgenic rat hearts.

Taken together, the upregulation of metabolic genes might thus represent a compensatory mechanism for an energetically inefficient myofibrillar contraction. Given that left ventricular morphology or function is not altered in DEL-TNT rats in MRI studies, these alterations in gene expression are not secondary to hypertrophic growth or heart failure but appear to be an early characteristic of disease pathogenesis, again suggesting that altered energy homeostasis might be an important feature of HCM.

Ventricular arrhythmias in DEL-TNT rats

HCM-associated mutations in cardiac troponin T are associated with a high incidence of sudden death, even in the absence of significant left ventricular hypertrophy [28]. In isolated working heart preparations from transgenic rats, we have previously observed an increased susceptibility to severe ventricular arrhythmias [13]. We now extended these findings by monitoring heart rate and rhythm in DEL-TNT transgenic rats in vivo. Since arrhythmic death in HCM patients particularly occurs on exertion and stress [29], we subjected transgenic rats and controls to increasing dosages of isoproterenol, a drug that also causes significant metabolic stress [20]. Again, DEL-TNT rats were more prone to develop ventricular arrhythmias upon isoproterenol stimulation, including severe arrhythmias such as ventricular tachycardia and fibrillation. Arrhythmias in HCM are commonly attributed to an increase in left ventricular muscle mass [30], myocyte disarray [28], and fibrosis [31]. However, at least in animal models of HCM, there is no clear correlation between the extent of cardiac fibrosis or myocyte disarray and the arrhythmic risk [32]. In our model, transgenic hearts do neither reveal left ventricular hypertrophy nor increased fibrosis [13], but nevertheless are susceptible to ventricular arrhythmias.

Recent experimental data on transgenic rats expressing the I79N-TNT mutation suggested that alterations in calcium cycling/homeostasis could contribute to ventricular arrhythmias in HCM [33]. Cardiomyocytes from these rats show depressed and prolonged Ca^{2+} transients compared to WT controls, which may trigger delayed afterdepolarizations and/or spontaneous Ca^{2+} oscillations [33]. This was associated with differential activation of the Ca^{2+}-dependent Na^{+}/Ca^{2+} exchanger. Slightly prolonged time parameters of Ca^{2+} transients have also been detected in ventricular trabeculae of DEL-TNT rats as well as a decreased maximum calcium activated force [14]. Thus, the increased energetic cost of force production caused by the DEL-TNT mutation [14] might both influence the force and the time response of the cross-bridge duty cycle. Given the fact that the activity of sarcoplasmatic reticulum Ca^{2+}-ATPase (SERCA) is highly energy dependent, a relative ATP depletion in DEL-TNT cardiomyocytes could trigger alterations of diastolic Ca^{2+} removal. In line with this, a decreased ratio of SERCA/phospholamban was found in transgenic rats expressing a TNT mutation with deletion of amino acid 160 [34].

Taken together, our data and findings from other authors imply that alterations in cardiac energy homeostasis could contribute to the development of ventricular arrhythmias in HCM caused by various TNT as well as other sarcomeric protein mutations. However, it seems likely that other factors such as fibrosis and severe hypertrophy play an additional role in arrhythmogenesis in affected patients.

In conclusion, we demonstrate that DEL-TNT rats reveal a significant dysregulation of cardiac energy metabolism, even in the absence of contractile dysfunction or significant hypertrophy. Inefficient sarcomeric energy utilization with increased myofibrillar ATP consumption could account for the compensatory upregulation of several metabolic genes as well as an increased mitochondrial density. It is therefore conceivable that cardiac energy supply might become limiting upon increased metabolic demand. Consistent with this notion, we observed contractile dysfunction in DEL-TNT cardiomyocytes subjected to metabolic stress. Likewise, transgenic rats revealed an increased susceptibility to isoproterenol-induced arrhythmias compared to wild-type controls.

Thus, our findings further support the “energy hypothesis” for HCM, which might provide a molecular basis for diastolic dysfunction and ventricular arrhythmias upon exercise in patients with hypertrophic cardiomyopathy. Future studies should evaluate if modulation of compromised cardiac energy metabolism may provide a new therapeutic approach for HCM.

Limitations of the study

Although the present study provides strong evidence for the contribution of dysregulated cardiac energy metabolism to the pathogenesis of HCM both in vitro and in vivo, the exact mechanisms by which mutations in sarcomeric proteins such as troponin T cause an energetically inefficient sarcomeric contraction on the molecular level still remain to be defined. Moreover, due to limitations of the rodent MRI setup, it was not possible to directly assess the metabolic state of the transgenic animals under conditions of stress such as exercise.

Acknowledgements The authors thank Ulrike Oehl and Jutta Krebs for their excellent technical support.

Conflict of Interest None declared

Funding N.F. was supported by the Bundesministerium für Bildung und Forschung, Germany (Nationales Genomforschungsnetz: NGFN2 and NGFNplus: 01650836), U.F. and J.S. were supported by the SFB 612 "Molekulare Analyse kardiovaskulärer Funktionen and Funktionsstörungen", Teilprojekt Z2, and the DFG project SCHR154/9.

References

1. Ashrafian H, Watkins H (2007) Reviews of translational medicine and genomics in cardiovascular disease: new disease taxonomy and therapeutic implications cardiomyopathies: therapeutics based on molecular phenotype. J Am Coll Cardiol 49:1251–1264
2. Arad M, Seidman JG, Seidman CE (2002) Phenotypic diversity in hypertrophic cardiomyopathy. Hum Mol Genet 11:2499–2506
3. Thierfelder L, Watkins H, MacRae C, Lamas R, McKenna W, Vosberg HP, Seidman JG, Seidman CE (1994) Alpha-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere. Cell 77:701–712
4. Sata M, Ikebe M (1996) Functional analysis of the mutations in the human cardiac beta-myosin that are responsible for familial hypertrophic cardiomyopathy. Implication for the clinical outcome. J Clin Invest 98:2866–2873
5. Lowey S (2002) Functional consequences of mutations in the myosin heavy chain at sites implicated in familial hypertrophic cardiomyopathy. Trends Cardiovasc Med 12:348–354
6. Moolman JC, Corfield VA, Posen B, Ngumbela K, Seidman C, Brink PA, Watkins H (1997) Sudden death due to troponin T mutations. J Am Coll Cardiol 29:549–555
7. Tobacman LS, Nihli M, Butters C, Heller M, Hatch V, Craig R, Lehman W, Homsher E (2002) The troponin tail domain promotes a conformational state of the thin filament that suppresses myosin activity. J Biol Chem 277:27636–27642
8. Montgomery DE, Tardiff JC, Chandra M (2001) Cardiac troponin T mutations: correlation between the type of mutation and the nature of myofilament dysfunction in transgenic mice. J Physiol 536:583–592
9. Ashrafian H, Redwood C, Blair E, Watkins H (2003) Hypertrophic cardiomyopathy: a paradigm for myocardial energy depletion. Trends Genet 19:263–268
10. Crilley JG, Boehm EA, Blair E, Rajagopalan B, Blamire AM, Styles P, McKenna WJ, Ostman-Smith I, Clarke K, Watkins H (2003) Hypertrophic cardiomyopathy due to sarcomeric gene mutations is characterized by impaired energy metabolism irrespective of the degree of hypertrophy. J Am Coll Cardiol 41:1776–1782
11. Tanaka T, Sohmiya K, Kawamura K (1997) Is CD36 deficiency an etiology of hereditary hypertrophic cardiomyopathy? J Mol Cell Cardiol 29:121–127
12. Merante F, Tein I, Benson L, Robinson BH (1994) Maternally inherited hypertrophic cardiomyopathy due to a novel T-to-C transition at nucleotide 9997 in the mitochondrial tRNA(glycine) gene. Am J Hum Genet 55:437–446
13. Frey N, Franz WM, Gloeckner K, Degenhardt M, Muller M, Muller O, Merz H, Katus HA (2000) Transgenic rat hearts expressing a human cardiac troponin T deletion reveal diastolic dysfunction and ventricular arrhythmias. Cardiovasc Res 47:254–264
14. Frey N, Brixius K, Schwinger RH, Benis T, Karpowski A, Lorenzen HP, Luedde M, Katus HA, Franz WM (2006) Alterations of tension-dependent ATP utilization in a transgenic rat model of hypertrophic cardiomyopathy. J Biol Chem 281:29575–29582
15. Flogel U, Jacoby C, Godecke A, Schrader J (2007) In vivo 2D mapping of impaired murine cardiac energetics in NO-induced heart failure. Magn Reson Med 57:50–58
16. Winer J, Jung CK, Shackel I, Williams PM (1999) Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytes in vitro. Anal Biochem 270:41–49
17. Tardiff JC, Factor SM, Tompkins BD, Hewett TE, Palmer BM, Moore RL, Schwartz S, Robbins J, Leinwand LA (1998) A truncated cardiac troponin T molecule in transgenic mice suggests multiple cellular mechanisms for familial hypertrophic cardiomyopathy. J Clin Invest 101:2800–2811
18. Vega RB, Huss JM, Kelly DP (2000) The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. Mol Cell Biol 20:1868–1876
19. Czubryt MP, McAnally J, Fishman GI, Olson EN (2003) Regulation of peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1 alpha) and mitochondrial function by MEF2 and HDAC5. Proc Natl Acad Sci USA 100:1711–1716
20. Hasenfuss G, Mulieri LA, Allen PD, Just H, Alpert NR (1996) Influence of isoproterenol and ouabain on excitation–contraction coupling, cross-bridge function, and energetics in failing human myocardium. Circulation 94:3155–3160
21. Redwood CS, Moolman-Smook JC, Watkins H (1999) Properties of mutant contractile proteins that cause hypertrophic cardiomyopathy. Cardiovasc Res 44:20–36
22. Sweeney HL, Feng HS, Yang Z, Watkins H (1998) Functional analyses of troponin T mutations that cause hypertrophic cardiomyopathy: insights into disease pathogenesis and troponin function. Proc Natl Acad Sci USA 95:14406–14410
23. Spindler M, Saupe KW, Christe ME, Sweeney HL, Seidman CE, Seidman JG, Ingwall JS (1998) Diastolic dysfunction and altered energetics in the alphaMHC403/+ mouse model of familial hypertrophic cardiomyopathy. J Clin Invest 101:1775–1783
24. Javadpour MM, Tardiff JC, Pinz I, Ingwall JS (2003) Decreased energetics in murine hearts bearing the R92Q mutation in cardiac troponin T. J Clin Invest 112:768–775
25. Sakata K, Ino H, Fujino N, Nagata M, Uchiyama K, Hayashi K, Konno T, Inoue M, Kato H, Sakamoto Y, Tsubokawa T, Yamagishi M (2008) Exercise-induced systolic dysfunction in patients with non-obstructive hypertrophic cardiomyopathy and mutations in the cardiac troponin genes. Heart 94:1282–1287
26. Shimizu M, Ino H, Yamaguchi M, Terai H, Uchiyama K, Inoue M, Ikeda M, Kawashima A, Mabuchi H (2003) Autopsy findings in siblings with hypertrophic cardiomyopathy caused by Arg92Trp

mutation in the cardiac troponin T gene showing dilated cardiomyopathy-like features. Clin Cardiol 26:536–539
27. Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP (2000) Peroxisome proliferator-activated receptor gamma coactivator-1 promotes cardiac mitochondrial biogenesis. J Clin Invest 106:847–856
28. Varnava AM, Elliott PM, Baboonian C, Davison F, Davies MJ, McKenna WJ (2001) Hypertrophic cardiomyopathy: histopathological features of sudden death in cardiac troponin T disease. Circulation 104:1380–1384
29. Maron BJ, Fananapazir L (1992) Sudden cardiac death in hypertrophic cardiomyopathy. Circulation 85:I57–I63
30. Spirito P, Bellone P, Harris KM, Bernabo P, Bruzzi P, Maron BJ (2000) Magnitude of left ventricular hypertrophy and risk of sudden death in hypertrophic cardiomyopathy. N Engl J Med 342:1778–1785
31. Marian AJ, Roberts R (2001) The molecular genetic basis for hypertrophic cardiomyopathy. J Mol Cell Cardiol 33:655–670
32. Wolf CM, Moskowitz IP, Arno S, Branco DM, Semsarian C, Bernstein SA, Peterson M, Maida M, Morley GE, Fishman G, Berul CI, Seidman CE, Seidman JG (2005) Somatic events modify hypertrophic cardiomyopathy pathology and link hypertrophy to arrhythmia. Proc Natl Acad Sci USA 102:18123–18128
33. Knollmann BC, Kirchhof P, Sirenko SG, Degen H, Greene AE, Schober T, Mackow JC, Fabritz L, Potter JD, Morad M (2003) Familial hypertrophic cardiomyopathy-linked mutant troponin T causes stress-induced ventricular tachycardia and Ca^{2+}-dependent action potential remodeling. Circ Res 92:428–436
34. Chandra M, Tschirgi ML, Tardiff JC (2005) Increase in tension-dependent ATP consumption induced by cardiac troponin T mutation. Am J Physiol Heart Circ Physiol 289:H2112–H2119