Journal of the American Heart Association

ORIGINAL RESEARCH

Cardiac Magnetic Resonance Reveals Incipient Cardiomyopathy Traits in Adult Patients With Phenylketonuria

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BACKGROUND: Phenylketonuria is the most common inborn error of amino acid metabolism, where oxidative stress and collateral metabolic abnormalities are likely to cause cardiac structural and functional modifications. We aim herein to characterize the cardiac phenotype of adult subjects with phenylketonuria using advanced cardiac imaging.

METHODS AND RESULTS: Thirty-nine adult patients with phenylketonuria (age, 30.5±8.7 years; 10-year mean phenylalanine concentration, 924±330 µmol/L) and 39 age- and sex-matched healthy controls were investigated. Participants underwent a comprehensive cardiac magnetic resonance and echocardiography examination. Ten-year mean plasma levels of phenylalanine and tyrosine were used to quantify disease activity and adherence to treatment. Patients with phenylketonuria had thinner left ventricular walls (septal end-diastolic thickness, 7.0±17 versus 8.8±1.7 mm [P<0.001]; lateral thickness, 6.1±1.4 versus 6.8±1.2 mm [P=0.004]), more dilated left ventricular cavity (end-diastolic volume, 87±14 versus 80±14 mL/m^2 [P=0.0178]; end-systolic volume, 36±9 versus 29±8 mL/m^2 [P<0.001]), lower ejection fraction (59±6% versus 64±6% [P<0.001]), reduced systolic deformation (global circumferential strain, –29.9±4.2 % versus –32.2±5.0 % [P=0.027]), and lower left ventricular mass (38.2±7.9 versus 47.8±11.0 g/m^2 [P<0.001]). T1 native values were decreased (936±53 versus 996±26 ms [P<0.001]), with particular low values in patients with phenylalanine >1200 µmol/L (909±48 ms). Both mean phenylalanine (P=0.013) and tyrosine (P=0.035) levels were independently correlated with T1; and in a multiple regression model, higher phenylalanine levels and higher left ventricular mass associate with lower T1.

CONCLUSIONS: Cardiac phenotype of adult patients with phenylketonuria reveals some traits of an early-stage cardiomyopathy. Regular cardiology follow-up, tighter therapeutic control, and prophylaxis of cardiovascular risk factors, in particular dyslipidemia, are recommended.

Key Words: cardiac magnetic resonance ■ cardiomyopathy ■ dyslipidemia ■ phenylketonuria ■ T1 native

With an incidence of ≈1:10 000 to 1:15 000, phenylketonuria is the most common inborn error of amino acid metabolism,[1] which is characterized by a low activity of phenylalanine hydroxylase, an enzyme responsible for the conversion of the essential amino acid phenylalanine to tyrosine. Lack of phenylalanine hydroxylase activity in untreated individuals leads to severe structural brain damage, resulting in complex psychological alterations and mental retardation, seizures, and spasticity.[2–4] Since the institution of newborn screening strategies in 1960s[5] followed by immediately beginning a phenylalanine-low diet

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Supplementary Material for this article is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.120.020351

For Sources of Funding and Disclosures, see page 15.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- Adult patients with "classic" phenylketonuria, treated lifelong with a specific phenylalanine-restrictive diet, present cardiac changes with reduced myocardial mass, thin myocardial walls, mildly impaired systolic function, and structural modifications revealed by parametric mapping.
- Structural modifications observed in treated adult patients with phenylketonuria are related to mean plasma levels of phenylalanine and tyrosine, measured frequently, and averaged over a period of 10 years, suggesting a long-term toxic effect of these higher concentrations of phenylalanine and/or lower concentrations of tyrosine, at present accepted as valid therapeutic targets by the American and European guidelines in place.

What Are the Clinical Implications?

- As the nature of this modifications, which may indicate an early model of cardiomyopathy, is not clearly understood and neither are the long-term consequences of these modifications, in the light of these findings, cardiac follow-up of adult patients with phenylketonuria, ideally with cardiac magnetic resonance, and developing larger multicentric studies are recommended.

Nonstandard Abbreviations and Acronyms

E	transmitral flow velocity
e′	early diastolic tissue velocity
ECV	extracellular volume
LVM	left ventricular mass
WT	wall thickness

combined with phenylalanine-free amino acid supplementation,[6] excellent progress has been made in reducing the neuropsychological complications and ensuring a normal intellectual development in affected individuals. This diet should be followed by patients with phenylketonuria lifelong[7]; however, a good constant adherence during adulthood is sometimes difficult to achieve. Moreover, little is known about the long-term efficacy of the phenylketonuria diet and about putative consequences of this highly selective food intake supported by periodic intermittent phenylalanine-free amino acid supplementations.

A series of potential cardiovascular risk factors have been associated with the disease, triggered by persistent metabolic abnormalities or by special, restrictive diet. Several studies identified increased levels of oxidative species resulting from the abnormal cellular metabolism[8] as well as from weaker cellular antioxidative defense (in particular, patients with phenylketonuria have lower levels of selenium, ubiquinone-10, and L-carnitine).[9] Corollary, increased levels of proinflammatory plasma cytokines, such as interleukin-6 and interleukin-1β, contribute to protein oxidation and lipid peroxidation.[10] Most studies agree that adult patients with phenylketonuria frequently display a certain degree of dyslipidemia[11] but not necessarily overt obesity or insulin resistance,[12] despite their vegan diet, in principle antiatherogenic.[6] Several studies found previously lower high-density lipoprotein (HDL) cholesterol concentrations[13,14] and higher concentrations of low-density lipoprotein (LDL) cholesterol[15,16] and triacylglycerol.[13] Recently, a vascular phenotype consisting of endothelial dysfunction and increased arterial stiffness, measured noninvasively with ultrasound, was identified in adult patients with phenylketonuria.[16] To date, no study evaluated comprehensively the cardiac phenotype in adults with phenylketonuria.

Cardiac magnetic resonance (CMR) is the current gold standard to evaluate cardiac dimensions and function.[17] In addition, novel protocols, based on parametric mapping, provide crucial information about cardiac structural modifications, such as interstitial fibrosis, inflammation, and substrate accumulation in the extracellular space.[18]

Herein, we characterized a cohort of adult patients with classic phenylketonuria, using advanced CMR and echocardiography, and compared their cardiac phenotype with that of age- and sex-matched healthy individuals.

METHODS

Because of the sensitive nature of the data collected for this study, requests to access the clinical data set from external researchers, directly interested in this study and group of patients, trained in human subject confidentiality protocols may be sent to the Interdisciplinary Center for Metabolism, Charité Universitätsmedizin Berlin at swc@charite.de. For the CMR protocols or other details related to the cardiovascular protocol, please contact the Deutsches Herzzentrum Berlin at info@dhzb.de.

Study Population

The study was approved by the Ethics Committee of the Charité Universitätsmedizin Berlin, complied with the Declaration of Helsinki, and was registered at the German Register for Clinical Studies

(DRKS00001120). A total number of 43 adult patients with an established diagnostic of phenylketonuria were recruited prospectively from the Interdisciplinary Center for Metabolism: Endocrinology, Diabetes and Metabolism of Charité University Berlin. The following inclusion criteria were used: aged >18 years, absence of any generic contraindication for CMR (claustrophobia, cardiac pacemakers or defibrillators, metallic implants, such as artificial valves or cerebral aneurysm clips, and morbid obesity), and signed consent to participate in all the stages of our protocol. Patients with any cardiac pathology (more than trivial valve disease, uncontrolled systemic hypertension, coronary artery disease, previous myocardial infarction, and cardiac arrhythmias) were excluded from the study. Four patients were excluded because of incomplete compliance with the protocol. Thus, 39 patients completed all the stages of our protocol, and their data were available for the functional analysis of the cardiac phenotype. A complete set of parametric imaging of sufficient quality to characterize the myocardial phenotype was obtained in 36 of 39 patients. For comparison, an age- and sex-matched group of 39 volunteers who underwent a similar protocol was included in the study. Controls were investigated without the administration of contrast medium and, therefore, T1 postcontrast and extracellular volume (ECV) could not be assessed directly. Controls were selected from our volunteer database at German Heart Center Berlin and partially characterized previously.[19,20]

Cardiac Magnetic Resonance

All CMR images were acquired using 1.5-T (Achieva; Philips Healthcare, Best, the Netherlands) magnetic resonance imaging scanners dedicated with a 5-channel cardiac surface coil in a supine position. All study participants were scanned using an identical comprehensive imaging protocol. The study protocol included initial scouts to determine cardiac imaging planes. Cine images were acquired using an ECG-gated balanced steady-state free precession sequence with multiple breath holds at end expiration in 3 left ventricular (LV) long-axis (2-, 3-, and 4-chamber) planes. The ventricular 2- and 4-chamber planes were used to plan a stack of short-axis slices covering the entire LV. The following imaging parameters were used: for 1.5-T scanner: repetition time=3.3 ms, echo time=1.6 ms, flip angle=60°, voxel size=1.8×1.7×8.0 mm^3, and 50 phases per cardiac cycle in accordance with standards of procedure established in our unit and described previously.[21]

Native and 15-minutes postcontrast T1 mapping studies were performed using a modified Look-Locker inversion-recovery at the level of 3 midventricular short-axis slices. Typical imaging parameters were as follows: acquired voxel size=2.0×2.0×10 mm^3, reconstructed voxel size=0.5×0.5×10 mm^3, balanced steady-state free precession readout, flip angle=35°, parallel imaging (sensitivity-encoding) factor=2, and effective inversion times between 150 and 3382 ms, as described previously.[20] Patients received 0.15 mmol/kg of gadolinium-based contrast agent gadoterate meglumine (Dotarem produced by Guerbet LLC, Princeton, NJ). Segmented inversion-recovery fast gradient–echo imaging was used to assess late gadolinium enhancement, 10 minutes after the administration of contrast substance.[22]

Image Analysis

All images were analyzed offline using commercially available software (Medis Suite, version 3.1; Leiden, the Netherlands) in accordance to recent consensus document for quantification of LV function using CMR.[23] LV short-axis epicardial and endocardial borders were manually contoured at end diastole and end systole to estimate LV mass and volumes. Left atrial (LA) volumes were assessed using a biplane area length method after segmentation of atrial contour in long-axis 4- and 2-chamber views and exclusion of LA appendage. Strain analysis included 2-, 3-, and 4-chamber cine images, and respectively, 3 preselected slices from the LV short-axis stack to correspond to basal, midventricular, and apical levels. LV, right ventricular, and LA strain were obtained by transferring the contours to QStrain RE version 2.0, where, after the application of tissue tracking algorithm, endocardial and epicardial borders were detected throughout all the cardiac cycle. Long-axis cine images (2, 3, and 4 chambers) were used to compute global myocardial longitudinal strain and, respectively, short-axis images (3 slices from basal, midventricular, and apical levels) were used to compute global circumferential strain. The global values for LV were obtained through averaging the values according to an American Heart Association 17 segments using a bull-eye view algorithm. Wall thickness (WT) was measured on the midventricular short-axis slice at end diastole, internal LV cavity diameter was measured basally at the level of papillary muscles at end diastole, and the maximal value was taken as result. Aortic distensibility was estimated at the level of ascending aorta by segmenting the systolic and diastolic section areas in 4-chamber cine images, as previously described.[24] Mapping parameters were measured using QMap RE version 2.0 (Medis Medical Imaging Systems bv, Leiden, the Netherlands). For parametric imaging, precontrast and postcontrast modified Look-Locker inversion-recovery images were adjusted for in-plane motion and then T1 native

and postcontrast relaxation times were determined using nonlinear fitting with a maximum likelihood estimator.[20] ECV was computed from precontrast and postcontrast T1 and hematocrit values, as described previously.[25] The extent of late gadolinium enhancement was defined as 2 SDs above the mean signal intensity of the distant myocardium and assessed in all the slices of short-axis stack for every subject with phenylketonuria.

Echocardiography

Transthoracic echocardiographic imaging was performed using an iE33 system with the X5–1 probe (Philips Healthcare, Best, the Netherlands). To characterize parameters related to the valvular and diastolic function, early (E) and late transmitral flow (A) and E/A ratio were assessed. Doppler tissue imaging was used to assess the velocity of paramitral segments (e′) and calculate E/e′ ratio, an imaging parameter evaluating noninvasively LV filling pressures. Echocardiography was performed by one dedicated cardiologist (M.G.) in all patients and controls.

Biochemistry

During the visits, routine hematological and biochemical parameters were determined. Median values of phenylalanine and tyrosine plasma concentrations were calculated using all the data available, covering a period of ≈10 years before the date of the CMR scan, obtained from the Interdisciplinary Center for Metabolism of the Charite University Berlin. These average concentrations were based on at least 20 single determinations (range, 20–60 determinations for each patient with phenylketonuria). Phenylalanine and tyrosine concentrations were measured in dried blood spots using mass spectrometry. In addition, data were obtained in the same laboratory unit, including the most recent serum levels of total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, carnitine-free fraction, and total glucose. All determinations were performed in duplicate by the hospital laboratory as part of the routine clinical evaluation of the patients (Labor Berlin—Charité Vivantes GmbH.)

Statistical Analysis

Statistical analysis was performed with IBM SPSS version 26. Normality of variables was assessed by visual assessment of normality curves and the Shapiro-Wilk test. Comparison between groups for continuous variables was performed with a 2-sided, independent-sample Student *t* test for normally distributed data and Mann-Whitney and Kruskal-Wallis tests for skewed data. For categorical variables, comparison was done with a χ^2 test, and statistical significance was evaluated with the Fisher exact test.

Univariate and bivariate regression models with T1 native as dependent variable and phenylalanine and tyrosine as predictors were used. To further test the dependency of T1 native on phenylalanine levels, we built a stepwise multiple regression with T1 native as dependent variable, and phenylalanine levels as independent, controlled for several clinically relevant covariates: plasma concentrations of lipids, LV mass, ejection fraction (EF), age, and sex, and having criteria for entry 0.05 and for removal 0.10. Unstandardized regression coefficients (B) were reported for univariate and bivariate regression models, and unstandardized and standardized coefficients (B) were reported for multiple regression model. To compare the 3 subgroups of patients with phenylketonuria divided according to the phenylalanine concentrations, a 1-way ANOVA for normally distributed data and, respectively, Kruskal-Wallis for nonnormally distributed data were performed followed by Tukey post hoc tests to compare differences among subgroups. Results are presented as mean±SD. Values of $P<0.05$ were considered statistically significant.

RESULTS

Population Characteristics

A complete set of these values is presented in Table 1. There was an equivalent distribution of comorbidities between the phenylketonuria and the control group. Patients with phenylketonuria have a slightly higher body mass index when compared with the control group (25.7±5.0 versus 23.7±3.3 kg/m^2 [P=0.03]). Among the patients with phenylketonuria, 10-year median phenylalanine concentration was 924±330 µmol/L. However, 21 of 39 (54%) of the patients with phenylketonuria had poor adherence with phenylalanine values >900 µmol/L.[26] Moreover, 27 of 39 patients had a phenylalanine concentration above the critical concentration of 800 µmol/L, associated with high oxidative stress and tissue damage[27] and specific structural and functional cerebral modifications.[28] Of 39 patients with phenylketonuria, 17 (44%) had a certain degree of dyslipidemia, most frequently reduced HDL cholesterol levels (14 of 17 [82%]). In addition, elevated concentrations of triglycerides (8 of 17 [47%]) or LDL cholesterol (10 of 17 [59%]) were observed.

Cardiac Anatomical Features and Function in Patients With Phenylketonuria

All quantitative date are presented in Table 2. We primarily compared the phenylketonuria cohort and controls with respect to the size, structure, and function of the heart. Subjects with phenylketonuria had a more dilated heart than controls (end-diastolic volume,

Table 1. Characteristics of the Study Population

Characteristic	Patients With Phenylketonuria (n=39)	Controls (n=39)	*P* Value*
Demographics			
Age, y	30.5±8.7	31.3±8.9	0.43
Men, n (%)	23 (55)	21 (50)	0.82
Smokers, n (%)	11 (26)	12 (29)	0.98
Anthropometrics			
Height, cm	172±10	174±10	0.42
Weight, kg	76±17	72±14	0.19
BMI, kg/m^2	25.7±5.0	23.7±3.3	0.03†
BSA, m^2	1.86±0.37	1.86±0.21	0.99
Clinical			
Heart rate, bpm	64±9	62±11	0.39
Systolic BP, mm Hg	114±15	112±8	0.75
Diastolic BP, mm Hg	66±9	67±7	0.87
Pulse pressure, mm Hg	47±11	46±8	0.57
Biochemistry			
Phenylalanine mean, µmol/L	924±330		
Phenylalanine median, µmol/L	915±326		
Phenylalanine SD, µmol/L	200±80		
Tyrosine mean, µmol/L	80±23		
Tyrosine median, µmol/L	76±25		
Tyrosine SD, µmol/L	33±14		
Carnitin free, mg/dL	34.9±10.9		
Carnitin total, mg/dL	50.5±14.0		
Carnitin ratio free/total	0.71±0.16		
Cholesterol total, mg/dL	164±33		
HDL cholesterol, mg/dL	47.3±13.7		
LDL cholesterol, mg/dL	92.8±26.3		
Triglycerides, mg/dL	150±98		
Glucose, mg/dL	78±16		

Values as are given as mean±SD, unless stated otherwise. Phenylalanine indicates last 10 years average plasma phenylalanine; and tyrosine, last 10 years average plasma tyrosine. BMI indicates body mass index; BP, blood pressure; bpm, beats per minute; BSA, body surface area; HDL, high-density lipoprotein; and LDL, low-density lipoprotein.

**P* value: patients with phenylketonuria vs age- and sex-matched controls. *P* values were adjusted with the Bonferroni method.

87±14 versus 80±14 mL/m^2 [*P*=0.018]; end-systolic volume, 36±9 versus 29±8 mL/m^2 [*P*<0.001]) (Figure 1A and 1B) with thinner walls (septal WT, 7.0±17 versus 8.8±1.7 mm [*P*<0.001]; lateral WT, 6.1±1.4 versus 6.8±1.2 mm [*P*=0.042]) and an important reduction in LV mass (38.2±7.9 versus 47.8±11.0 g/m^2 [*P*<0.001]) (Figure 1G.). LV mass deficit remained significant even when indexed with end-diastolic volume (0.44±0.06 versus 0.61±0.12 g/mL [*P*<0.001]). LV EF (59±6% versus 64±6% [*P*<0.001]) and LV global circumferential strain (–29.9±4.2% versus –32.2±5.0% [*P*=0.027]) are reduced but not global longitudinal strain (Figure 1D through 1F), and E/A ratio was within normal limits, with an increased early transmitral flow velocity. LA volumes were not different from control, but LA emptying fraction and LA strain were increased, suggesting increased atrial pressures. Right ventricular EF was not significantly decreased; however, the means for right ventricular EF and right ventricular global longitudinal strain were lower in the phenylketonuria group (Table 2).

Parametric Imaging and Correlations With Plasma Phenylalanine Concentrations

Compared with control, T1 native relaxation time was shorter in the phenylketonuria group (936±53 versus 996±26 ms [*P*<0.001]) (Figure 1H). We did not administer contrast to the volunteers participating in the study, and for comparing ECV data, we used reference values, corresponding to the scanner model and protocol used in our center, published elsewhere.[29] There was no difference in the ECV between the patients with phenylketonuria and this reference value (26.5±4.4% versus 25.0±4.0% [*P*=0.15]) (Figure 1I).

Table 2. Cardiac Structure and Function in Patients With Phenylketonuria

Variable	Patients With Phenylketonuria (n=39)	Controls (n=39)	*P* Value*
LV			
ED volume, mL/m^2	87±14	80±14	0.0178†
ES volume, mL/m^2	36±9	29±8	<0.001†
Stroke volume, mL/m^2	51±8	51±8	0.83
Ejection fraction, %	59±6	64±6	<0.001†
Cardiac index, L/min/m^2	3.57±0.74	3.25±0.63	0.037†
Longitudinal strain, %	−25.1±3.1	−25.3±2.8	0.74
Circumferential strain, %	−29.9±4.2	−32.2±5.0	0.0273†
Septal ED WT, mm	7.0±17	8.8±1.7	<0.001†
Lateral ED WT, mm	6.1±1.4	6.8±1.2	0.042†
Relative WT	1.15±0.15	1.32±0.23	<0.001†
LV mass, g/m^2	38.2±7.9	47.8±11.0	<0.001†
LV mass/ED volume, g/mL	0.44±0.06	0.61±0.12	<0.001†
LV ED maximal diameter, mm	53±4	52±5	0.10
LA			
LA maximal volume, mL	37.2±8.0	35.1±9.3	0.29
LA minimal volume, mL	10.7±3.3	11.3±5.7	0.57
LA emptying fraction, %	71±5	67±8	0.0117†
LA strain, %	49.5±12.5	40.5±15.5	0.008†
RV			
RV maximal volume, mL	46.2±11.2	42.2±10.7	0.11
RV minimal volume, mL	17.2±6.7	14.8±5.0	0.07
RV ejection fraction, %	62.7±7.9	65.4±6.7	0.11
RV longitudinal strain, %	−25.0±5.5	−29.0±5.7	0.0019†
Ascending aorta			
Systolic aortic diameter, mm	45.5±0.9	41.9±1.0	0.25
Diastolic aortic diameter, mm	35.2±0.9	32.7±0.8	0.38
Aortic distensibility, 10^{-3} mm Hg^{-1}	6.76±2.60	6.40±1.54	0.64
Echocardiography			
MV E Vmax, cm/s	80±14	71±17	0.02†
MV A Vmax, cm/s	52±13	61±19	0.046†
MV E/A	1.61±0.43	1.32±0.60	0.027†
E/e′	6.36±2.27	5.18±1.44	0.11
	Patients With Phenylketonuria (n=36)	**Controls (n=39)**	***P* Value***
Parametric imaging			
T1 native, ms	936±53	996±26	<0.001†
T1 after contrast, ms	592±45		
ECV, %	26.5±4.4	25.0±4.0‡	0.15

Values as are given as mean±SD. e' indicates early diastolic velocity of basal paramitral segments; ECV, extracellular volume; ED, end diastolic; ES, end systolic; LA, left atrium; LV, left ventricle; MV, mitral valve; MV A Vmax, late (atrial) diastolic transmitral flow velocity; MV E Vmax, early diastolic trsnmitral flow velocity; RV, right ventricle; WT, wall thickness.

**P* value: patients with phenylketonuria vs age- and sex-matched controls. All *P* values were adjusted with the Bonferroni method.

†A *P*<0.05 was considered statistically significant.

‡Mean and SD referenced in literature (Dabir et al, *J Cardiovasc Magn Reson*. 2014;16:69) for similar scanner and magnetic field strength.

T1 values were negatively correlated with phenylalanine (*r*=−048; *P*=0.002) (Figure 2A) and positively correlated with tyrosine (*r*=0.44; *P*=0.005) (Figure 2B), suggesting that shorter, more abnormal, T1 values associate with poor dietary adherence among patients with phenylketonuria. To establish the individual and cumulative effect of phenylalanine and tyrosine concentrations for T1 values, we performed univariate and bivariate multiple regression, and results are presented in Figure 3C.

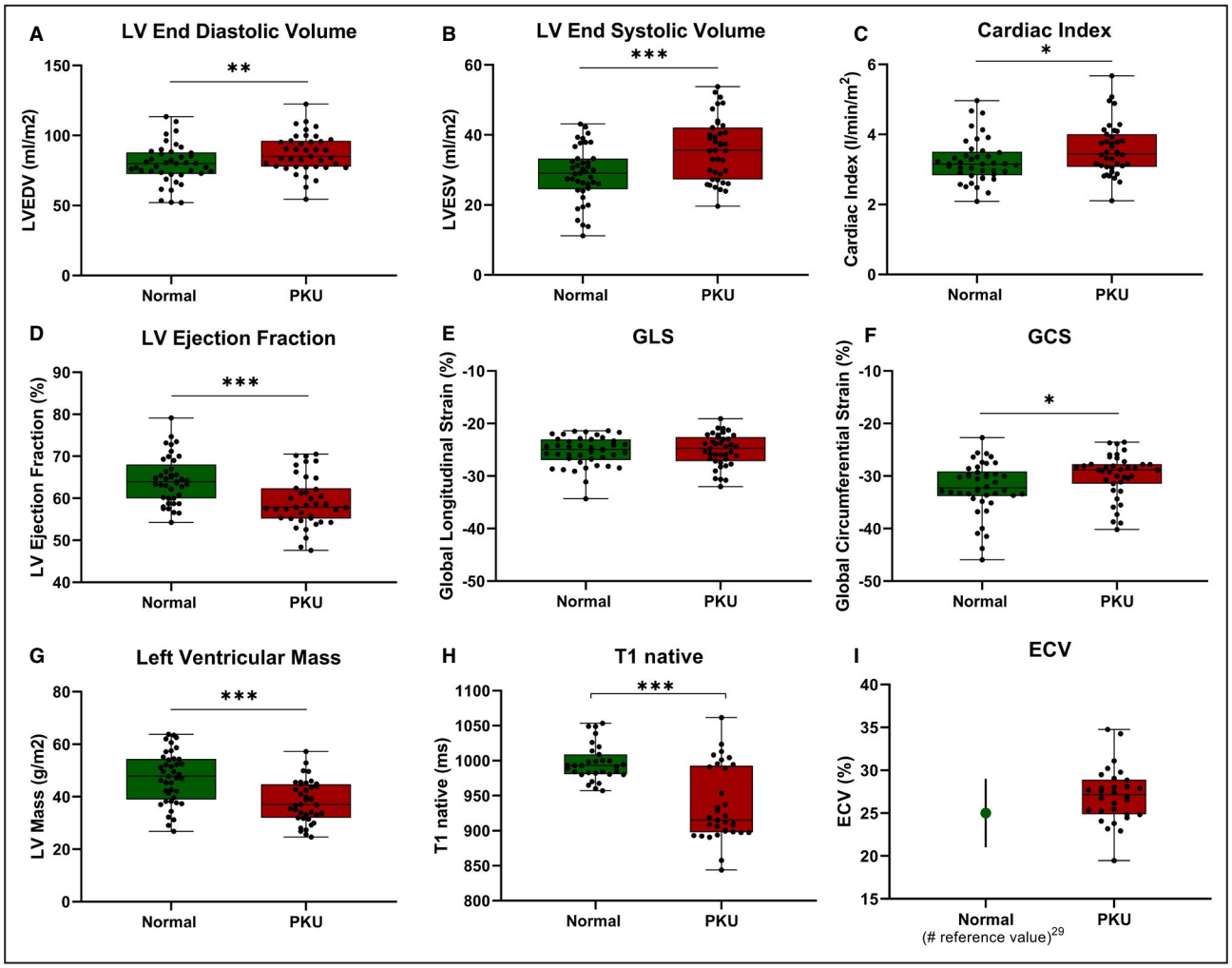


Figure 1. Cardiac structural and functional remodeling in adult patients with phenylketonuria (PKU) and age- and sex-matched controls.

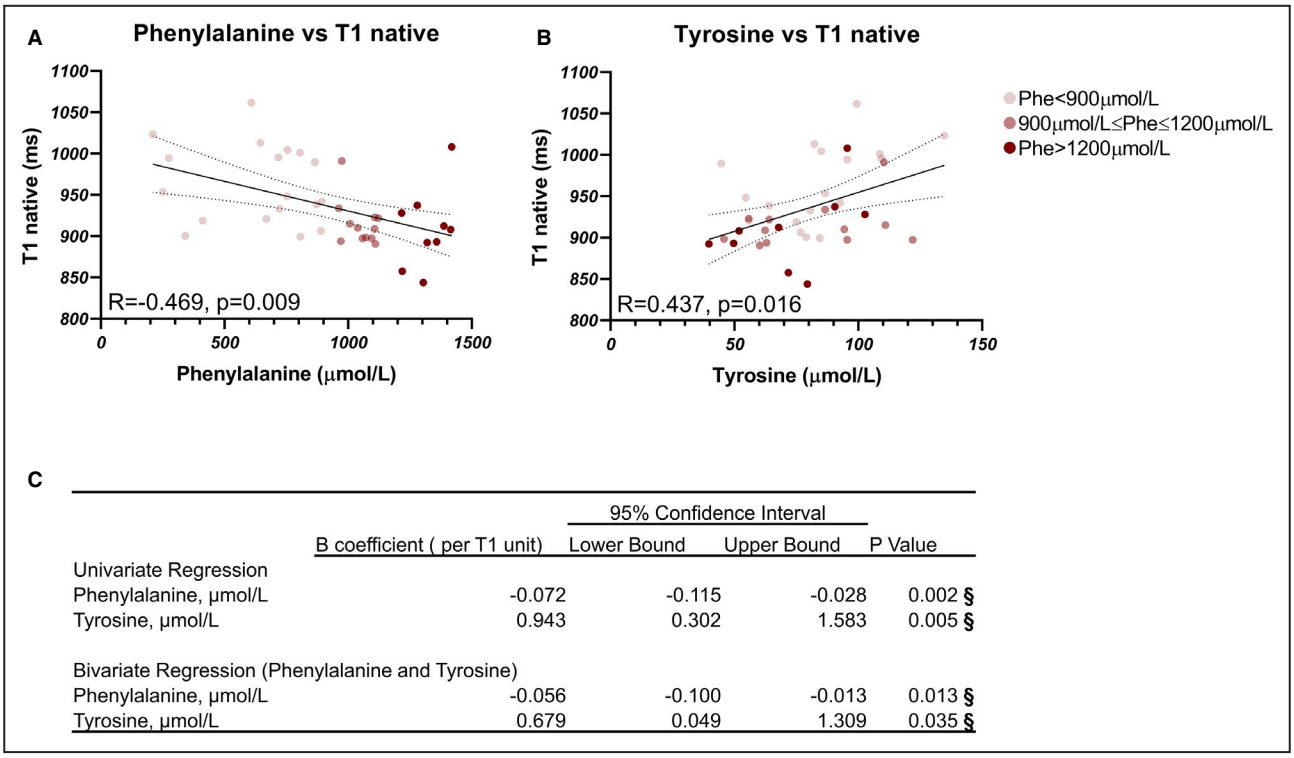
Left ventricular end-diastolic volume (LVEDV) (**A**), left ventricular end-systolic volume (LVESV) (**B**), cardiac index (**C**), left ventricular (LV) ejection fraction (**D**), global longitudinal strain (GLS) (**E**), global circumferential strain (GCS) (**F**), LV mass (**G**), T1 native (**H**), and extracellular volume (ECV) (**I**) (number mean and SD referenced in literature [Dabir et al, *J Cardiovasc Magn Reson*. 2014;16:69][29] for similar scanner and magnetic field strength). A $P<0.05$ was considered statistically significant. *$P<0.05$, **$P<0.01$, and ***$P<0.001$.

On the other hand, T1 values correlated negatively with LV mass (LVM) ($r=-0.50$; $P=0.004$) (Figure 2C), septal WT ($r=-0.52$; $P=0.001$), and lateral WT ($r=-0.49$; $P=0.001$), indicating that lower relaxation times correspond to an increase in mass and WT of the myocardium. There was no association between T1 values and parameters characterizing LV systolic function, but T1 was correlated with E/e′ ratio ($R=-0.487$; $P=0.004$), suggesting that an incipient LV impaired lusitropy may be related to these presumed structural abnormalities. In a multivariable regression model, T1 as dependent variable was predicted by phenylalanine (standardized β, −0.34; $P=0.024$) and LVM (standardized β, −0.43; $P=0.005$) with a resultant $R=0.59$, $P=0.001$ (Figure 3).

To test any association with the cardiac phenotype, we compared the lipid plasma concentrations with structural and functional parameters. Total cholesterol ($r=-0.40$; $P=0.021$) and HDL fraction ($r=-0.45$; $P=0.009$), but not LDL fraction or triglyceride concentration, correlated inversely with LVM. Taken together, these findings indicate that reduced levels of HDL cholesterol, which are most frequently seen in subjects with phenylketonuria, may play an important role to promote dyslipidemia and, thus, enhance lipid accumulation in the heart.

Analysis of Subgroups

To test if persistently higher concentrations of phenylalanine influenced the cardiac structure and function in the patients, we divided the phenylketonuria cohort according to phenylalanine levels recorded



C

		95% Confidence Interval		
	B coefficient (per T1 unit)	Lower Bound	Upper Bound	P Value
Univariate Regression				
Phenylalanine, μmol/L	-0.072	-0.115	-0.028	0.002 §
Tyrosine, μmol/L	0.943	0.302	1.583	0.005 §
Bivariate Regression (Phenylalanine and Tyrosine)				
Phenylalanine, μmol/L	-0.056	-0.100	-0.013	0.013 §
Tyrosine, μmol/L	0.679	0.049	1.309	0.035 §

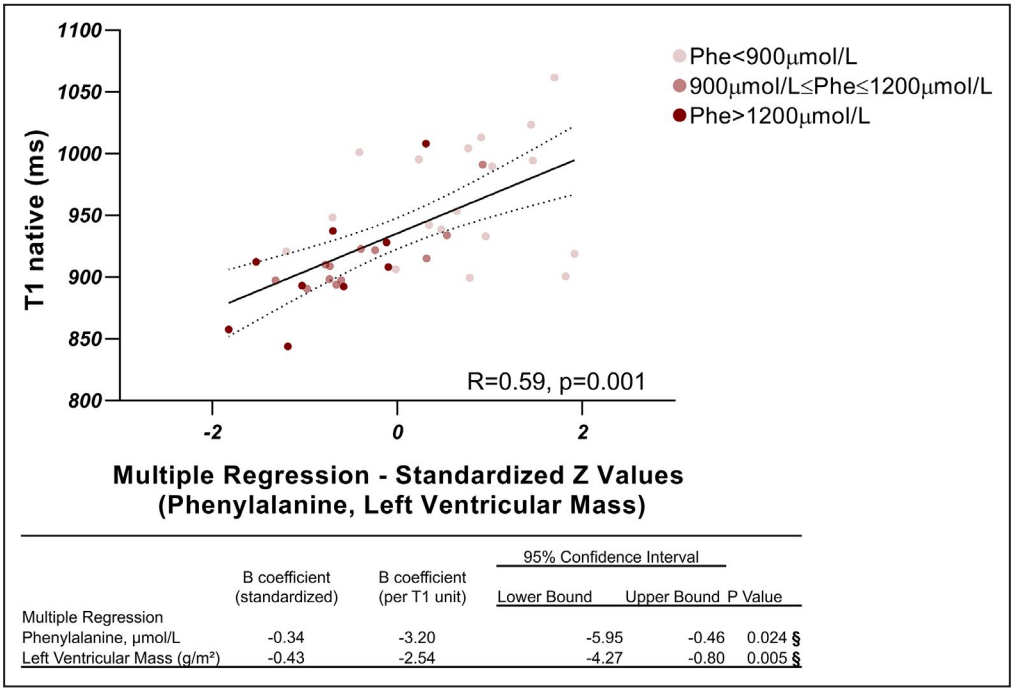
Figure 2. Univariate and bivariate regression to determine the relation between 10-year average levels of phenylalanine (Phe) and tyrosine (Tyr) and T1 native values.
A, Linear regression between Phe plasma levels and T1. **B**, Linear regression between Tyr plasma levels and T1 (continuous line represents the regression best fit equation line, and the dotted lines represent the 95% confidence limits). **C**, Regression parameters and B coefficients. A $P<0.05$ was considered statistically significant; Phe and Tyr plasma levels predict independently T1 values, indicating that therapeutic intervention not only to reduce Phe levels but also to augment the Tyr levels may be beneficial to correct cardiac changes. § indicates statistical significance.

before the CMR scan into 3 subgroups, as follows: patients with phenylketonuria with phenylalanine <900 μmol/L, patients with phenylalanine between 900 and 1200 μmol/L, and patients with phenylalanine >1200 μmol/L (data presented in Table 3). There were no significant differences between these 3 subgroups in total, HDL, or LDL cholesterol; however, in patients with a poorer control of the disease (phenylalanine >1200 μmol/L), triglycerides and LDL/HDL ratio were higher than in patients with optimal control (phenylalanine <900 μmol/L). Detailed data are given in Table 3 and represented in Figure 4. Age is only modestly correlated with total (R^2=0.21; P=0.004), HDL (R^2=0.15; P=0.019), and LDL (R^2=0.13; P=0.027) cholesterol but not with triglycerides and LDL/HDL ratio (Figure S1) or with phenylalanine levels.

Of 39 patients, 21 (54%) had phenylalanine values >900 μmol/L, and 9 of 39 (23%) patients had phenylalanine values >1200 μmol/L. Parameters characterizing cardiac size and systolic function were not different between the subgroups; however, there were some trends in the means of several of these parameters, such as a tendency to higher thickness of the cardiac walls and LV diameters in the subgroups characterized by higher phenylalanine levels. T1 native values were comparatively lower (964±48 versus 915±27 versus 909±48 [P<0.003]) in subgroups with high phenylalanine levels, whereas ECV was comparable among the groups (Figure 5). E/e′, an echocardiographic biomarker of abnormal LV filling pressures and diastolic dysfunction, was higher in patients with higher phenylalanine (5.24±1.62 versus 5.87±1.57 versus 8.33±1.74 [P<0.001]).

More important, within-subject phenylalanine level SD recorded for the same time frame as the median (on average, 10 years) correlated positively with both end-diastolic volume (R=0.357; P=0.026) and end-systolic volume (R=0.359; P=0.025). This would suggest that a tighter control of phenylalanine values over time may be efficient in preventing adverse LV remodeling seen in subjects with phenylketonuria.

Similarly, to establish if abnormal concentrations of lipids were correlated with cardiac phenotype, we compared a subgroup of the patients with phenylketonuria with at least one abnormal lipid plasma level (normal-range reference values were considered: total cholesterol, <200 mg/dL; HDL



	B coefficient (standardized)	B coefficient (per T1 unit)	95% Confidence Interval Lower Bound	Upper Bound	P Value
Multiple Regression					
Phenylalanine, μmol/L	-0.34	-3.20	-5.95	-0.46	0.024 §
Left Ventricular Mass (g/m²)	-0.43	-2.54	-4.27	-0.80	0.005 §

Figure 3. In a multivariate regression model, the independent predictors of T1 native are the averaged levels of plasma phenylalanine (Phe) (B coefficient standardized, −0.34) and respectively left ventricular mass (B coefficient standardized, −0.43), both negatively correlated with T1 native times.
We suggest that the pathologic substrate that may be responsible for lower T1 times is more present in the myocardium tissue of patients with worse control of the disease. § indicates statistical significance.

cholesterol, >45 mg/dL; LDL cholesterol, <130 mg/dL; and triglycerides, <180 mg/dL) and respectively a subgroup composed of patients with normal levels of lipids. These data are presented in Table 4. In the dyslipidemic group, LV walls were thicker (septal WT, 7.8±2.0 versus 6.4±1.2 mm [*P*=0.011]; lateral WT, 6.9±1.5 versus 5.6±1.1 mm [*P*=0.003]) and the cavity was significantly more dilated (end-diastolic volume, 93±12 versus 83±14 mL/m^2 [*P*=0.0019]; end-systolic volume, 40±10 versus 33±7 mL/m^2 [*P*=0.012]), but EF was equivalent. However, there is a trend that global longitudinal strain, global circumferential strain, and EF are all lower in the dyslipidemic than in the normolipidemic subgroup.

DISCUSSION

Our findings can be summarized as follows:

1. Patients with phenylketonuria had thinner cardiac walls, a more dilated LV, and a lower myocardial mass than their healthy counterparts.
2. A functional deficit was also notable, with significantly reduced values of LV EF and circumferential strain on CMR and mildly elevated E/e′ on Doppler echocardiogram.
3. In patients with phenylketonuria, T1 native was significantly shorter than in matched controls.
4. Plasma levels of phenylalanine and tyrosine were independently correlated with reduced T1 values, strongly supporting the idea that a stricter control of these values can be successful in preventing pathologic structural remodeling. In a multiple regression model, phenylalanine and LVM were both negatively predictive for T1 values.
5. Dyslipidemic patients with phenylketonuria had more dilated LV cavity and thicker LV walls, reinforcing the hypothesis that an abnormal lipid profile, possibly associated with other metabolic dysfunctions or oxidative stress, may be responsible for the observed cardiac changes.

This investigation demonstrates cardiac abnormalities in adult patients with phenylketonuria. The thinner and more dilated heart of the patient with phenylketonuria likely represents a relatively early stage of dilated cardiomyopathy.[30] Even if within generically normal range, the EF was reduced compared with age-matched individuals. Moreover, global circumferential strain was also reduced in patients with phenylketonuria. Because of the structural preponderance of the circumferentially distributed fibers, systolic strain

Table 3. Cardiac Structure and Function in Patient Subgroups With Phenylketonuria, Divided According to 10-Year Mean Phenylalanine Plasma Concentrations: Phenylalanine ≤900 µmol/L, 900 µmol/L<Phenylalanine≤1200 µmol/L, and Phenylalanine >1200 µmol/L

Variable	Phenylalanine ≤900 µmol/L (n=18)	900 µmol/L <Phenylalanine≤1200 µmol/L (n=12)	Phenylalanine >1200 µmol/L (n=9)	*P* ANOVA	*P* Value*	*P* Value†	*P* Value‡
LV							
ED volume, mL/m^2	84±14	92±13	86±14	0.31			
ES volume, mL/m^2	35±9	37±9	37±10	0.75			
Stroke volume, mL/m^2	50±8	56±8	49±7	0.09			
Ejection fraction, %	59±6	61±6	57±6	0.45			
Longitudinal strain %	−25.4±3.4	−25.6±2.3	−23.9±3.3	0.41			
Circumferential strain %	−30.3±4.1	−30.1±3.6	−28.9±4.0	0.69			
Cardiac index	3.4±0.6	3.9±0.7	3.5±1.0	0.27			
Septal ED WT, mm	6.7±1.7	6.9±1.6	7.8±1.9	0.26			
Lateral ED WT, mm	5.8±1.4	6.1±1.1	6.8±1.7	0.26			
Relative WT, mm	1.16±0.15	1.13±0.15	1.16±0.15	0.82			
LV mass, g/m^2	36.7±8.3	40.1±6.7	38.5±8.7	0.52			
LV mass/ED volume, g/mL	0.43±0.07	0.44±0.06	0.45±0.06	0.90			
LV ED maximal diameter, mm	52±5	53±4	56±4	0.10			
LA							
LA maximal volume, mL/m^2	37±6	39±7	34±12	0.37			
LA minimal volume, mL/m^2	11±3	11±3	10±4	0.29			
LA emptying fraction, %	70±6	73±4	73±5	0.37			
LA strain, %	49.1±15.3	51.0±9.8	48.0±9.6	0.87			
RV							
RV maximal volume, mL/m^2	45±10	49±14	45±11	0.51			
RV minimal volume, mL/m^2	17±7	18±7	18±5	0.97			
RV ejection fraction, %	62±9	64±8	61±6	0.65			
RV longitudinal strain, %	−25.2±4.8	−26.5±4.9	−22.5±7.2	0.26			
Ascending aorta							
Systolic aortic diameter, mm	46.2±9.4	44.2±9.2	45.2±10.1	0.81			
Diastolic aortic diameter, mm	36.0±9.4	33.4±8.3	35.7±10.3	0.91			
Aortic distensibility, 10^{-3} mm Hg^{-1}	7.96±2.99	6.48±1.84	6.48±3.04	0.39			
Echocardiography							
MV E Vmax, cm/s	75±12	80±14	80±13	0.52			
MV A Vmax, cm/s	49±13	54±7	50±15	0.56			
MV E/A	1.64±0.38	1.51±0.38	1.70±0.48	0.50			
E/e′	5.24±1.62	5.87±1.57	8.33±1.74	<0.001§	0.62	<0.001§	0.001§
Plasma lipids							
Total cholesterol, mg/dL	155±31	161±31	173±43	0.47			
Trigylcerides, mg/dL	126±57	113±83	241±111	0.002§	0.91	0.004§	0.003§
HDL cholesterol, mg/dL	52±14	48±10	42±13	0.17			
LDL cholesterol, mg/dL	86±19	94±28	99±31	0.41			
LDL/HDL cholesterol ratio	1.70±0.34	2.03±0.68	2.54±0.82	0.008§	0.32	0.006§	0.15

(*Continued*)

Table 3. Continued

	Phenylalanine ≤900 µmol/L (n=18)	900 µmol/L <Phenylalanine≤1200 µmol/L (n=9)	Phenylalanine >1200 µmol/L (n=9)				
Parametric imaging							
T1 native	964±48	915±27	909±48	0.003§	0.012§	0.001§	0.94
T1 after contrast	591±56	613±33	577±25	0.25			
ECV, %	27±9	27±4	26±2	0.72			

Values as are given as mean±SD. *P* ANOVA, ANOVA between the 3 groups, divided according to 10-year mean value of plasma phenylalanine concentrations: group 1, phenylalanine ≤15 mg/dL; group 2, 15 mg/dL<phenylalanine≤20 mg/dL; and group 3, phenylalanine >20 mg/dL. e' indicates early diastolic velocity of basal paramitral segments; ECV, extracellular volume; ED, end diastolic; ES, end systolic; HDL, high-density lipoprotein; LA, left atrium; LDL, low-density lipoprotein; LV, left ventricle; MV, mitral valve; MV A Vmax, late (atrial) diastolic transmitral flow velocity; MV E Vmax, early diastolic trsnmitral flow velocity; RV, right ventricle; and WT, wall thickness.

**P* value: comparison between group 1 and group 2.

†*P* value: comparison between group 1 and group 3.

‡*P* value: comparison between group 2 and group 3. All *P* values were adjusted with the Bonferroni method.

§A *P*<0.05 was considered statistically significant.

at this level is responsible for most of the resultant ejection force.[31] Although longitudinally distributed fibers are more sensitive to inflammation, microvascular dysfunction, or metabolic alterations, a selective reduction in circumferential deformation is typically observed in dilated cardiomyopathies with an intrinsic contractile deficit.[32] Aortic distensibility and diastolic function were within the normal parameters in subjects with phenylketonuria; however, a comparatively worse, higher E/e′ ratio was seen in poorly controlled compared with well-controlled patients.

On the other hand, paradoxically increased cardiac index (Figure 1C), coupled with a relation between the lipid abnormal concentrations and ventricular dilatation, suggests that the pathophysiological model of dysfunction in phenylketonuria has some common traits with the one found in obesity cardiomyopathy.[33] Indeed, as previously documented,[34] our patients had a higher body mass index compared with controls. In contrast with cardiac remodeling observed in obese subjects, however, myocardial mass was severely reduced in patients with phenylketonuria. Intriguingly, in subjects with increased LV mass (ie, closer to the normal mean), this could be attributable to structural changes, such as potential intramyocardial substrate deposition or infiltration rather than myocyte hypertrophy.

Cardiovascular risk in patients with phenylketonuria may result from the cumulative effects of abnormal production of toxic metabolites, in particular free oxygen species, intermittent nutritional deficiencies resulting from specific diet intake, and, in some cases, episodes of incompliant behavior and poor metabolic control of the disease. Recent studies confirm increased plasma levels of oxidative stress markers and resultant specific vascular effects, such as arterial stiffness[35] and endothelial dysfunction[16] in patients with phenylketonuria. Within the cardiac tissue, oxidative stress is responsible for depressed contractility, myocyte apoptosis, and interstitial and focal fibrotic deposition.[36] We could not identify any increased CMR marker of focal or interstitial fibrosis; however, we observed shorter myocardial T1 native times in most patients with phenylketonuria and in particular in the subgroups with an intermediate and poor adherence to diet (900 µmol/L≤phenylalanine≤1200 µmol/L and phenylalanine >1200 µmol/L). LVM and WT correlated negatively with T1; and in a multiple regression model, phenylalanine levels and LVM are, in tandem, independent negative predictors for T1. Even if globally reduced when compared with normal subjects, in patients with phenylketonuria, an increase in LVM likely represents an adverse structural remodeling of the heart. To date, although multiple cardiac disease have been associated with T1 native prolongation, shorter T1 is observed only in lipid accumulation (typically seen in cardiomyopathy associated with Anderson-Fabry disease) or cardiac siderosis.[37] Long-standing lipid profile modifications coupled with other metabolic abnormalities (such as deficit in carnitine), a more pronounced LV dilatation, and hypertrophy make us speculate that an intramyocyte deposition of lipids[38] may be at least in part responsible for the structural modifications detected by parametric mapping. The precise nature or substrate of these changes is yet to be established by further studies, in particular histological analysis on biopsy samples or heart tissue from animal models of treated phenylketonuria. An intriguing alternative hypothesis would be that higher plasma levels of phenylalanine depose in the myocardium, as cytotoxic amyloid-like fibrils, in a similar pattern to those identified in brain tissue

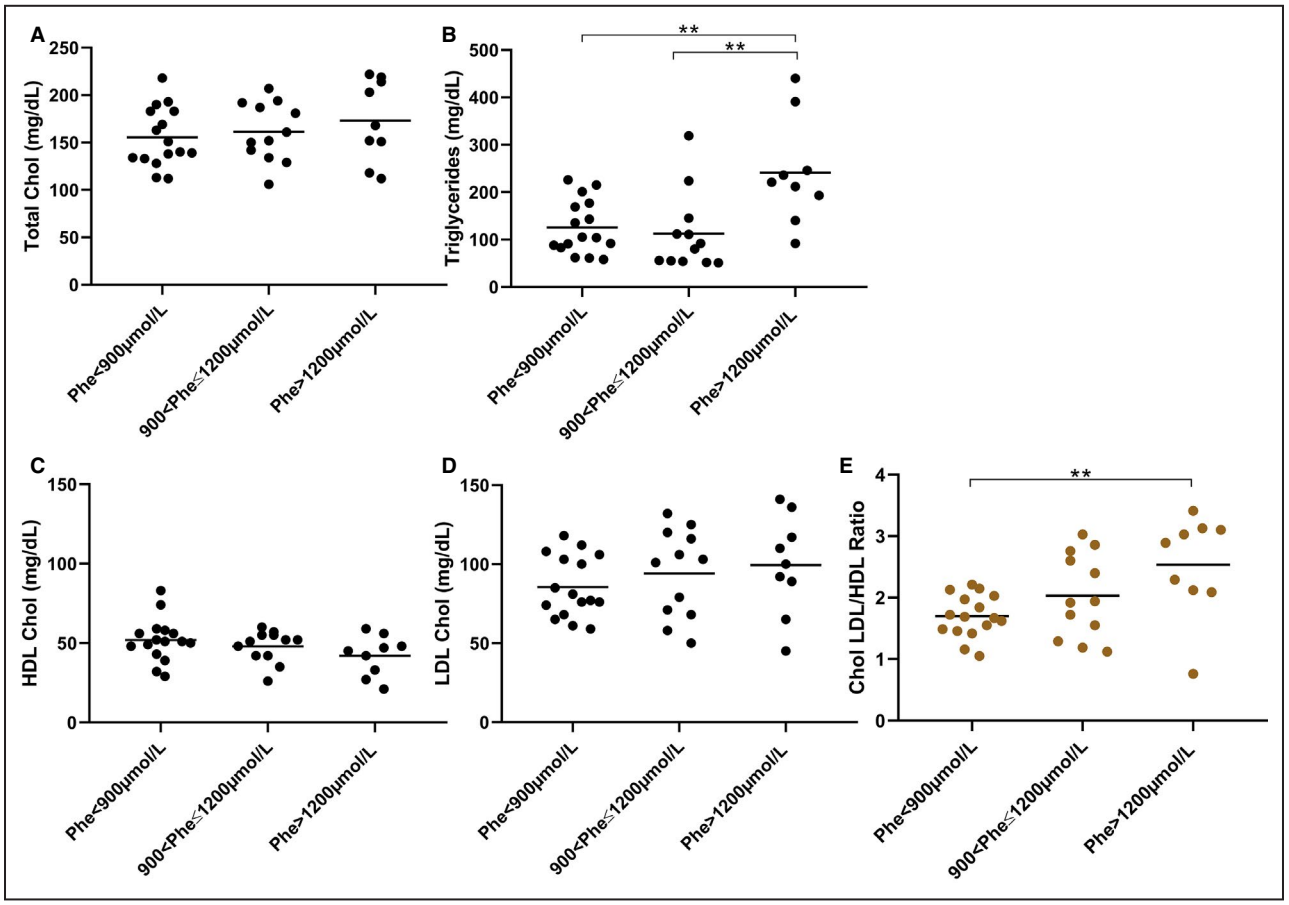


Figure 4. Lipid profile in 3 subgroups of adult patients with phenylketonuria, divided according to the 10-year mean phenylalanine (Phe) level (from left to right in each graph: group 1, Phe ≤900 μmol/L; group 2, 900 μmol/L<Phe≤1200 μmol/L; and group 3, Phe >1200 μmol/L).

Total cholesterol (Chol) (**A**), triglycerides (**B**), high-density lipoprotein (HDL) cholesterol (**C**), low-density lipoprotein (LDL) cholesterol (**D**), and LDL/HDL cholesterol ratio (**E**). Significant differences were recorded between the level of triglycerides and LDL/HDL cholesterol ratio, with a more pronounced dyslipidemic profile observed in patients with poor control of the disease (ie, higher mean Phe values).

from patients with phenylketonuria and transgenic animal models.[39]

In other pathological conditions, such as Anderson-Fabry disease, aortic stenosis,[40] or diabetes mellitus,[41] cardiac steatosis has a progressive negative impact on the mechanical function of the heart.[42] With this study, we did not manage to show conclusively a relationship between lower T1 values and reduced mechanical contraction (assessed by myocardial strain or EF). However, given the incipient dilative pattern seen in patients with phenylketonuria with an abnormal lipid profile, our data suggest that this may be a plausible outcome in the evolution of this disease, in particular in patients with more pronounced lipid abnormalities. In the Framingham cohort, both increased LDL and decreased HDL cholesterol are risk factors for developing heart failure, independent of coexistence of coronary artery.[43] In addition, prolonged dyslipidemic abnormalities, frequently present in adults with phenylketonuria,[44] and identified in our cohort, may determine per se a progressive worsening of cardiac function.[45]

Limitations

Phenylketonuria is a rare disease in which metabolic control and avoiding neuropsychological complications have been to date the clinical priorities. In consequence, our proof-of-principle study was limited in size, exploratory, and cross-sectional. We could not establish a direct correlation between the deficit in cardiac contraction and structural modifications, such as decreased T1 values, or between lipid plasma concentrations and T1 values. In the absence of clinical indication for this procedure, myocardial samples for histological analysis were not available and, therefore, the

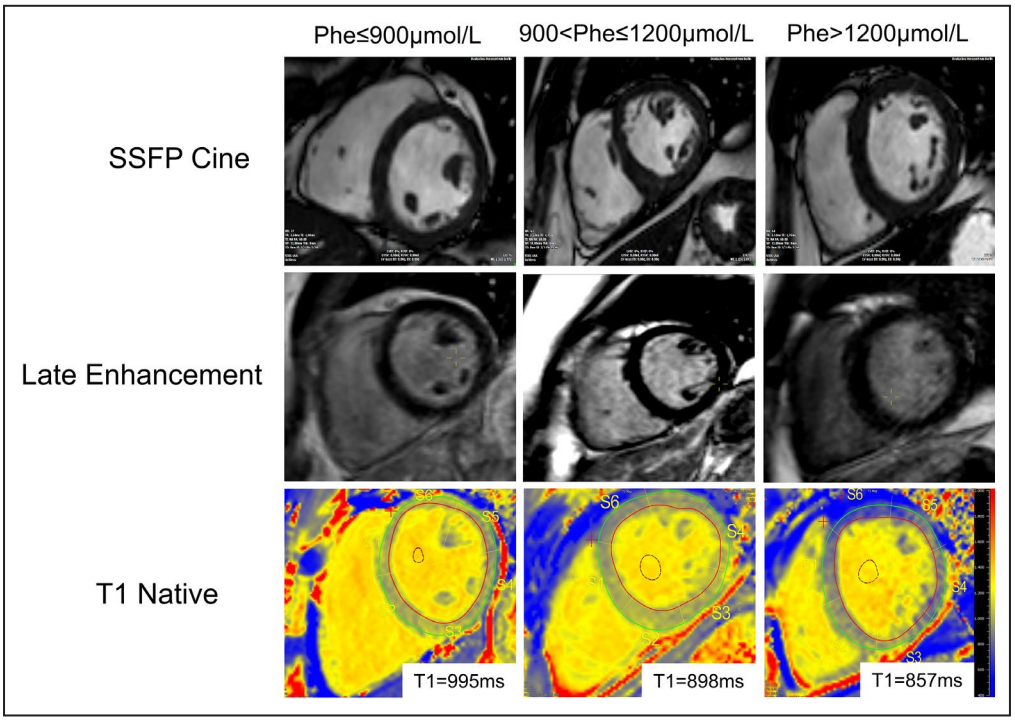


Figure 5. Representative steady-state free precession (SSFP) cine (first row), late gadolinium enhancement (middle row), and T1 native maps (lower row) of 3 subjects with phenylketonuria from 3 groups, divided according to mean 10-year value of plasma phenylalanine (Phe) concentrations: group 1, Phe ≤900 μmol/L; group 2, 900 μmol/L<Phe≤1200 μmol/L; and group 3, Phe >1200 μmol/L, displayed as follows: group 1 subject (left column), group 2 subject (middle column), and group 3 subject (right column).
In the absence of any focal lesion, there is a diffuse process that shortens T1 native times in patients in whom the therapeutic control of Phe is suboptimal.

nature of structural changes leading to abnormal T1 remains to be established first by animal studies. Apart from phenylalanine levels, an indirect indicator of compliance with the recommended restrictive diet, our analysis did not include more specific details on various other amino acid levels, prophylactic food supplements, and nutrition status of these patients; therefore, we could not exclude the possibility that complex metabolic abnormalities and their interaction are responsible for these cardiac changes. To elucidate these aspects, further studies, including systematic proteomic and lipidomic approaches, are warranted. Information about potential tissue inflammation and edema, given by CMR tools, such as T2 mapping,[20] is missing for this cohort. The blood tests available for the patients with phenylketonuria at the time of the initial CMR scan did not include biomarkers of cardiac dysfunction, such as NT-proBNP (N-terminal pro-B-type natriuretic peptide) or cardiac troponin T, with a proven role in early detection of myocardial impairment[46,47] that more recently has become part of routine evaluation in cardiovascular medicine. We anticipate that, with an increased age range, as was available in this study, these correlations will become apparent. We would also expect more pronounced modification of the cardiac phenotype at a follow-up visit. At this stage, larger multicentric longitudinal studies are warranted to further test these presumptions.

CONCLUSIONS

Adult patients with phenylketonuria present a mild form of cardiomyopathy, with dilated heart with thinner ventricular walls and reduced LVM. Reduced T1 values likely reflect pathological intramyocardial substrate deposition, which has the potential to contribute further to flare up chronic inflammation, induce interstitial fibrosis, and further decrease contractility. We advocate that regular follow-up of these patients should include frequent echocardiograms or, ideally, CMR evaluation, in particular in cases where concentrations of phenylalanine remain high or the compliance with the specific diet is reduced.

Table 4. Cardiac Structure and Function in Patient Subgroups With Phenylketonuria, Divided According to Lipid Plasma Concentrations: Normolipidemic and Dyslipidemic

Variable	Normolipidemic Phenylketonuria (n=22)	Dyslipidemic Phenylketonuria (n=17)	*P* Value*
LV			
ED volume, mL/m^2	83±14	93±12	0.019†
ES volume, mL/m^2	33±7	40±10	0.012†
Stroke volume, mL/m^2	50±9	53±7	0.23
Ejection fraction, %	60±5	58±7	0.16
Longitudinal strain, %	−25.9±2.5	−24.0±3.5	0.052
Circumferential strain, %	−30.7±3.7	−28.9±4.6	0.2
Cardiac index, L/min/m^2	3.6±0.8	3.6±0.6	0.89
Septal ED WT, mm	6.4±1.2	7.8±2.0	0.011†
Lateral ED WT, mm	5.6±1.1	6.9±1.5	0.003†
Relative WT	1.16±0.14	1.13±0.16	0.59
LV mass, g/m^2	36.1±0.06	40.8±8.2	0.065
LV mass/EDV, g/mL	0.44±0.06	0.44±0.07	0.96
LV ED diameter, mm	51±9	55±4	0.017†
LA			
LA maximal volume, mL/m^2	37±8	38±8	0.56
LA minimal volume, mL/m^2	10±3	11±4	0.48
LA emptying fraction, %	72±5	71±6	0.75
LA strain, %	49±13	50±13	0.79
RV			
RV maximal volume, mL/m^2	44±11	49±11	0.2
RV minimal volume, mL/m^2	16±7	20±6	0.079
RV ejection fraction, %	64±8	61±7	0.23
RV longitudinal strain, %	−25.3±5.1	−24.5±6.2	0.65
Ascending aorta			
Systolic aortic diameter, mm	43.8±7.7	47.7±10.7	0.20
Diastolic aortic diameter, mm	34.4±8.2	36.2±9.9	0.53
Aortic distensibility, 10^{-3} mm Hg^{-1}	6.36±2.47	7.26±2.73	0.29
Echocardiography			
MV E Vmax, cm/s	84±15	76±12	0.063
MV A Vmax, cm/s	55±14	49±11	0.18
MV E/A	1.60±0.49	1.61±0.34	0.96
E/e′	5.99±1.75	6.83±1.74	0.19
Plasma lipids			
Total cholesterol, mg/dL	161±32	163±37	0.85
Trigylcerides, mg/dL	131±108	174±70	0.15
HDL cholesterol, mg/dL	53±8	42±15	0.010†
LDL cholesterol, mg/dL	88±25	97±26	0.30
LDL/HDL cholesterol	1.69±0.53	2.43±0.61	<0.001†
	Normolipidemic Phenylketonuria (n=19)	**Dyslipidemic Phenylketonuria (n=17)**	
Parametric imaging			
T1 native	935±52	938±47	0.87
T1 after contrast	585±53	599±36	0.39
ECV, %	26.0±5.3	27.0±3.4	0.51

Values as are given as mean±SD. e' indicates early diastolic velocity of basal paramitral segments; ECV, extracellular volume; ED, end diastolic; EDV, ED volume; ES, end systolic; HDL, high-density lipoprotein; LA, left atrium; LDL, low-density lipoprotein; LV, left ventricle; MV, mitral valve; MV A Vmax, late (atrial) diastolic transmitral flow velocity; MV E Vmax, early diastolic trsnmitral flow velocity; RV, right ventricle; and WT, wall thickness.

**P* value: comparison between nondyslipidemic and lipidemic patients. *P* values were adjusted with the Bonferroni method.

†A *P*<0.05 was considered statistically significant.

ARTICLE INFORMATION

Received December 2, 2020; accepted May 17, 2021.

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Acknowledgment

Open access funding enabled and organized by ProjektDEAL.

Disclosures

Dr Pieske reports having received consultancy and lecture honoraria from Bayer Daiichi Sankyo, MSD, Novartis, Sanofi-Aventis, Stealth Peptides, and Vifor Pharma; and editor honoraria from the *Journal of the American College of Cardiology*. The remaining authors have no disclosures to report.

Sources of Funding

Dr Kelle is supported by a grant from Philips Healthcare and received funding from the German Centre for Cardiovascular Research and by the German Ministry of Education and Research.

Supplementary Material

Figure S1

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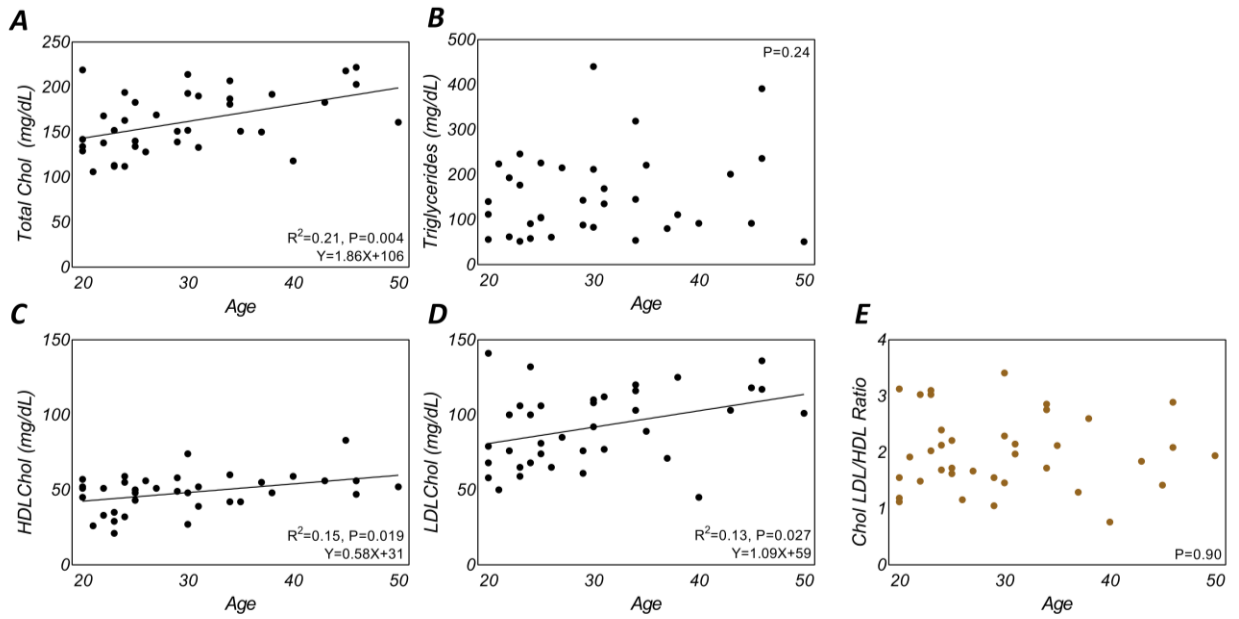
Supplemental Material

Figure S1. Age influence on lipid levels in Phenylketonuria patients.



A. Total Cholesterol, **B.** Triglycerides, **C.** HDL Cholesterol, **D.** LDL Cholesterol, **E.** LDL/HDL Cholesterol Ratio. Only total, HDL and LDL cholesterol are mildly correlated with age.

Abbreviations: HDL – high-density lipoprotein, LDL – low-density lipoprotein. HDL – high-density lipoprotein, LDL – low-density lipoprotein, Chol – cholesterol.