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Diastolic left ventricular function in relation to circulating metabolic biomarkers in a population study

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Abstract

Aims: We studied the association of circulating metabolic biomarkers with asymptomatic left ventricular diastolic dysfunction, a risk-carrying condition that affects 25% of the population.

Methods and results: In 570 randomly recruited people, we assessed in 2005–2010 and in 2009–2013 the multivariable-adjusted correlations of e' (early left ventricular relaxation) and E/e' (left ventricular filling pressure) measured by Doppler echocardiography with 43 serum metabolites, quantified by magnetic resonance spectroscopy. In 2009–2013, e' cross-sectionally increased (Bonferroni corrected $p \leq 0.016$) with the branched-chain amino acid valine (per one standard deviation increment, +0.274 cm/s (95% confidence interval, 0.057–0.491)) and glucose+the amino acid (AA) taurine (+0.258 cm/s (0.067–0.481)), while E/e' decreased ($p \leq 0.017$) with valine (–0.264 (–0.496– –0.031)). The risk of developing left ventricular diastolic dysfunction over follow-up (9.4%) was inversely associated ($p \leq 0.0059$) with baseline glucose+amino acid taurine (odds ratio, 0.64 (0.44–0.94). In partial least squares analyses of all the baseline and follow-up data, markers consistently associated with better diastolic left ventricular function included the amino acids 2-aminobutyrate and 4-hydroxybutyrate and the branched-chain amino acids leucine and valine, and those consistently associated with worse diastolic left ventricular function glucose+amino acid glutamine and fatty acid pentanoate. Branched-chain amino acid metabolism ($-\log10_p = 12.6$) and aminoacyl-tRNA biosynthesis (9.9) were among the top metabolic pathways associated with left ventricular diastolic dysfunction.

Conclusion: The associations of left ventricular diastolic dysfunction with circulating amino acids and branched-chain amino acids were consistent over a five-year interval and suggested a key role of branched-chain amino acid metabolism and aminoacyl-tRNA biosynthesis in maintaining diastolic left ventricular function.

Keywords

Biomarker, branched-chain amino acids, diastolic left ventricular dysfunction, metabolomics, population science

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Introduction

Over the past decade, metabolomics has developed into a state-of-the-art technology providing metabolic fingerprints of individual patients as a gateway to risk stratification, identification of molecular mechanisms and possibly personalised medicine.[1] The 2015 Global Burden of Disease report[2] stated that heart failure affects 40 m of the world's population. Several case-control studies compared circulating metabolic signatures between patients with advanced heart failure and normal controls[3,4] or between heart failure patients with preserved and reduced ejection fraction.[5,6] Landmark population studies[7] also introduced metabolomics as a novel tool in population research. However, population-based studies did not yet relate early-stage asymptomatic diastolic left ventricular (LV) dysfunction with circulating metabolic profiles. Asymptomatic diastolic LV dysfunction affects over 25% of the general population.[8,9] It carries a 10% risk for further deterioration over five years,[10] and is a forerunner of cardiovascular complications.[11] In a cross-sectional analysis of the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO),[12] using a non-targeted metabolomic approach, we previously demonstrated that diastolic LV function was associated with circulating metabolites indicative of energy substrate utilisation or protection against oxidative stress and that these metabolites differentiated normal function from asymptomatic diastolic LV dysfunction. In the present study, we investigated the consistency of these associations five years later and we explored to what extent the baseline levels of the circulating metabolites predicted diastolic LV function and the incidence of diastolic LV dysfunction at follow-up.

Methods

Design, participants, and setting

FLEMENGHO is a family-based population study representative for a defined geographic area in northern Belgium, for which recruitment started in 1985 and continued until 2004.[12] The initial participation rate was 78.0%. The study complies with the Helsinki declaration. The Ethics Committee of the University Hospitals Leuven approved the protocol. Participants were repeatedly followed up. Of 711 individuals,[12] who from 2005–2010 underwent a first echocardiographic examination along with an assessment of their circulating metabolites, 667 survivors still residing in the catchment area were eligible for a second assessment, of whom 575 renewed written informed consent (86.2%). Of those, we excluded five from analysis, because of atrial fibrillation ($n = 2$) or a paced heart rhythm ($n = 3$). Thus the number of participants with a full set of baseline (2005–2010) and follow-up (2009–2013) data totalled 570 (Supplementary Material Figure S1).

Clinical and biochemical measurements

At baseline (2005–2010) and follow-up (2009–2013) venous blood samples were drawn after ≥8 h of fasting. Body mass index was weight in kilograms divided by height in meters squared. Blood pressure was the average of five consecutive auscultatory readings. Hypertension was a blood pressure of ≥140 mm Hg systolic or ≥90 mm Hg diastolic or use of antihypertensive drugs. Mean arterial pressure was diastolic pressure plus one-third of the difference between systolic and diastolic pressure. Estimated glomerular filtration rate (eGFR) was derived from serum creatinine by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[13] Diabetes mellitus was a self-reported diagnosis, a fasting plasma glucose of 7 mmol/l or higher, or use of antidiabetic agents.

Echocardiography

A single observer acquired the echocardiographic images and did the off-line analysis according to current guidelines.[14] In continuous analyses, lower e' (early LV relaxation) and higher E/e' (LV filling pressure) indicated worse diastolic LV function. Patients with diastolic LV dysfunction had an abnormally low age-specific transmitral E/A ratio indicative of impaired relaxation or a mildly-to-moderately elevated LV filling pressure (E/e' >8.5) with normal or decreased age-specific E/A ratio.[8] These age-specific criteria, first established in a healthy reference sample of the FLEMENGHO study,[8] were subsequently replicated in an independent European cohort.[9]

Nuclear magnetic resonance spectroscopy

At baseline and follow-up, venous blood was sampled from an antecubital vein and immediately spun for 10 min at 1500 g in a refrigerated centrifuge. The supernatant plasma was stored for maximum two months at –20°C and afterwards at –80°C. For measurement of the metabolites, plasma samples were within 24 h shipped on dry ice from Leuven to Valencia and after arrival kept deep frozen at –80°C until analysis. We measured 43 metabolites in plasma using nuclear magnetic resonance (NMR) spectroscopy and methods described in previous publications[12,15] and in the Supplementary Material (pp. 2–3). To study reproducibility of the NMR measurements, the complete aliphatic spectral region was split into 0.005 ppm buckets. The mean bucket difference for all aliphatic

spectral regions was 5.1% with a maximum of 7.0% for the bucket containing the HDL apolipoprotein signal (Supplementary Material Figure S2). NMR spectroscopy is fast and keeps samples separated from the instrument, but produces crowded spectra that cannot always be reliably deconvoluted into single metabolites.[16] When two peaks contributed to a spectral region, the two metabolites were jointly reported.[12,15]

Statistical analysis

For database management and statistical analysis, we used SAS, version 9.4 (SAS Institute Inc., Cary, North Carolina, USA). Means were compared using the large-sample z-test or analysis of variance (ANOVA) and proportions the Fisher exact test. For pairwise comparison of proportions, we used the McNemar test. We normalised the distribution of γ-glutamyltransferase by a logarithmic transformation. We rank normalised the distributions of the metabolites by sorting measurements from the smallest to the highest and then applying the inverse cumulative normal function. We computed 95% confidence interval of rates as

$$R \pm 1.96 \times \sqrt{(R/T)}$$

where R and T are the rate and the denominator used to calculate the rate.

Using the partial regression coefficients derived by multiple linear regression, we standardised e' and E/e' to the population averages of covariables identified in previous publications,[12] including sex, age, body mass index, mean arterial pressure, heart rate, total serum cholesterol, serum γ−glutamyltransferase (as index of alcohol intake and potential predictor of heart failure[17]), fasting plasma glucose, LV mass index, treatment with diuretics, β−blockers and inhibitors of the renin-angiotensin system (angiotensin-converting enzyme inhibitors and angiotensin II type-1 receptor blockers). While accounting for covariables, including baseline values of the echocardiographic indexes in the longitudinal analyses, we regressed e' and E/e' on the metabolic markers. We applied mixed models to account for relatedness as a random effect and the other covariables as fixed effects. We used cluster analysis to classify correlated circulating metabolites into groups with the eigenvalue set at 1 (Supplementary Material pp. 3–4 and Table S1).[7] Based on the number of groups so determined, we adjusted significance levels and confidence intervals for multiple testing by the Bonferroni approach. In an attempt to differentiate direct associations of diastolic LV function (e' and E/e') with the metabolic markers from indirect associations mediated via LV mass index, we ran the PROC CALIS procedure, as implemented in SAS 9.4 (maintenance level TS1M1).

In the next step of our analyses, we applied partial least squares analysis (PLS) to identify a set of independent latent factors that were linear combinations of the metabolites and maximised the covariance between the variables describing diastolic LV function (e' and E/e') and the metabolic markers. We retained the smallest number of latent factors for which the predicted residual sums of squares (PRESS, calculated using the leave-one-out cross-validation) did not significantly differ ($p > 0.10$) from the model with the minimum PRESS value, as assessed by the van der Voet T^2 statistic. The importance of each metabolite in the construction of the PLS factors in relation to diastolic LV function was assessed from the variable importance in projection (VIP) score of Wold with the threshold set at 1.1.[12] Finally, we used metabolic pathway analysis to estimate the relative importance of metabolites associated with diastolic LV function and the pathways that they represent (MetPA, version 3.0, Edmonton, Canada).

Results

Baseline and follow-up characteristics of participants

All 570 participants were white Europeans (50.1% women). Age at baseline averaged 50.6 years (interquartile range (IQR), 42.2–59.8 years. Median follow-up was 4.7 years (IQR, 4.4–5.1 years). From baseline (2005–2010) to follow-up (2009–2013), blood pressure increased by 3.7 mm Hg systolic and 2.7 mm Hg diastolic, body mass index by 0.7 kg/m^2 and serum creatinine by 5.7 μmol/l, whereas eGFR decreased by 7.5 ml/min/1.73 m^2 ($p < 0.0001$; Table 1). Over follow-up, the prevalence of hypertension, treated hypertension, use of lipid-lowering drugs and diabetes mellitus all increased, whereas the number of people reporting smoking declined (Table 1). The 570 analysed participants had baseline characteristics, which were broadly similar to those of 141 individuals, who were examined at baseline but not followed up (Supplementary Material Table S2).

Over follow-up, all echocardiographic measurements changed significantly ($p < 0.0001$; Supplementary Material Table S3). Left atrial volume index increased by 2.8 ml/m^2 and LV mass index by 3.3 g/m^2. Transmitral E, A and E/A and mitral annular e', a', e'/a' decreased by 8.5 cm/s, 3.4 cm/s and 0.08 and by 1.7 cm/s, 0.6 cm/s and 0.11, respectively. The opposite was the case for E/e', which increased by 0.39 during follow-up (Supplementary Material Table S3). At baseline, LV ejection fraction was greater than 50% in all but three participants and from baseline to follow-up

Table 1. Baseline and follow-up characteristics of 570 participants.

Characteristic	Baseline 2005–2010	Follow-up 2009–2013	Change over time 95% CI
Number of participants (%)			
Women	287 (50.1)	287 (50.1)	. . .
Smokers	107 (18.7)	84 (14.7)	–4.0 (–5.9– –2.2)[a]
Drinking alcohol	240 (41.9)	221 (38.6)	–3.3 (–6.9–0.2)
Hypertension	231 (40.3)	294 (51.3)	11.1 (7.6–14.5)[a]
Antihypertensive treatment	137 (23.9)	182 (31.8)	7.9 (5.4–10.4)[a]
Lipid-lowering treatment	83 (14.6)	144 (25.3)	10.7 (7.8–13.6)[a]
Diabetes mellitus	18 (3.2)	34 (5.9)	2.8 (1.4–4.3)[b]
Mean of characteristic			
Age (years)	50.6 (14.6)	55.3 (14.5)	4.7 (4.6–4.7)[a]
Body mass index (kg/m^2)	26.6 (4.3)	27.3 (4.3)	0.7 (0.6–0.9)[a]
Blood pressure (mm Hg)			
Systolic pressure	128.6 (16.3)	132.3 (16.5)	3.7 (2.6–4.8)[a]
Diastolic pressure	79.9 (9.3)	82.5 (9.8)	2.7 (1.9–3.4)[a]
Mean arterial pressure	96.1 (10.2)	99.1 (10.0)	3.0 (2.3–3.8)[a]
Heart rate (beats/min)	60.8 (9.4)	60.6 (9.7)	–0.3 (-0.9–0.4)
Biochemical data			
Serum creatinine (μmol/l)	84.3 (16.4)	90.0 (23.5)	5.7 (4.5–7.0)[a]
eGFR (ml/min/1.73 m^2)	81.9 (16.2)	74.4 (15.5)	–7.5 (–8.3– –6.7)[a]
Total cholesterol (mmol/l)	5.24 (0.94)	5.00 (0.93)	–0.24 (–0.31– –0.16)[a]
Plasma glucose (mmol/l)	4.93 (0.76)	4.92 (0.72)	–0.01 (–0.08–0.06)
γ-Glutamyltransferase (units/l)	23 (16–33)	24 (16–33)	1.7 (0.7–2.8)[b]

CI: confidence interval; eGFR: estimated glomerular filtration rate (derived from serum creatinine by the Chronic Kidney Disease Epidemiology Collaboration equation).

Baseline and follow-up values are number of participants (%), arithmetic mean (standard deviation (SD)), or geometric mean (interquartile range). Changes are given in percentage for categorical variables and the logarithmically transformed γ-glutamyltransferase. Hypertension was a blood pressure of ≥140 mm Hg systolic or ≥90 mm Hg diastolic, or use of antihypertensive drugs. Mean arterial pressure is diastolic pressure plus one third of the difference between systolic and diastolic pressure. Diabetes mellitus was a self-reported diagnosis, a fasting glucose level of ≥7 mmol/l, or use of antidiabetic agents. Significance of the changes over time: [a]$p \leq 0.0001$; [b]$p \leq 0.001$.

decreased slightly but significantly ($p = 0.0058$) from 68.4% (standard deviation (SD), 6.8) to 67.5% (6.8%).

Associations with single metabolites

Table 2 lists the metabolites, which in multivariable cross-sectional analyses of the 2009–2013 data retained significance with adjustment for multiple testing applied. A one-SD increment in serum valine (branched-chain amino acid (BCAA)) was associated ($p \leq 0.017$) with higher e' (+0.274 cm/s) and lower E/e' (–0.264). Higher glucose+taurine (amino acid (AA)) was associated with greater e' (+0.258 cm/s; $p = 0.0066$) and higher 2-oxobutyrate (a short-chain fatty acid (SCFA)) with lower E/e' (–0.335; $p = 0.0012$). For the markers listed in Table 2, we differentiated direct associations with the index of diastolic LV function from indirect associations mediated by LV mass index. Considering e', direct/indirect correlations were 0.10 ($p = 0.015$)/0.033 ($p = 0.0025$) for valine, 0.13 ($p = 0.0014$)/–0.0046 ($p = 0.63$) for glucose + taurine, and –0.14 ($p = 0.064$)/–0.046 ($p = 0.012$) for pentanoate. Considering E/e', direct/indirect correlations were –0.092 ($p = 0.030$)/–0.028 ($p = 0.0050$) for valine and –0.11 ($p = 0.013$)/–0.033 ($p = 0.0024$) for 2-oxobutyrate.

In the longitudinal analysis, we correlated e' and E/e' at follow-up (2009–2013), while adjusting for their baseline value (e' or E/e') and for baseline covariables and metabolites (2005–2010). Per one-SD increment in the baseline biomarkers, e' at follow-up was 0.239 cm/s (CI, 0.008–0.471; $p = 0.040$) lower in relation to the SCFA pentanoate. E/e' at follow-up was 0.186 (CI, 0.006–0.365; $p = 0.040$) lower in relation to baseline glucose + AA taurine and 0.274 cm/s (CI, 0.043–0.505; $p = 0.011$) lower in relation to the baseline level of BCAA valine. Thus, the longitudinal associations with valine, pentanoate and glucose + taurine were directionally and statistically consistent with the cross-sectional associations of the 2009–2013 data (Table 2).

Table 2. Cross-sectional associations of e' and E/e' with the metabolites at follow-up.

Tissue Doppler Indexmetabolite	Estimate (95% CI)	p	p_B
e' peak			
Valine	0.274 (0.057–0.491)	0.0009	0.0054
Glucose + taurine	0.258 (0.067–0.481)	0.0011	0.0066
Pentanoate	–0.268 (–0.501– –0.035)	0.0026	0.016
E/e' ratio			
Valine	–0.264 (–0.496– –0.031)	0.0029	0.017
2-Oxobutyrate	–0.335 (–0.572– –0.098)	0.0002	0.0012

All estimates accounted for relatedness and were adjusted for sex, age, body mass index, mean arterial pressure, heart rate, total cholesterol, γ−glutamyltransferase, plasma glucose, left ventricular (LV) mass index and treatment with diuretics, β−blockers and inhibitors of the renin-angiotensin system. Estimates express the change in the dependent variable per one standard deviation (SD) increase in the circulating metabolites. P and P_B respectively refer to significance without and with correction applied for multiple testing. 95% Confidence intervals (CIs) account for multiple testing.

Of 435 people with normal diastolic LV function at baseline, 41 progressed to diastolic LV dysfunction (Supplementary Material Table S4). The incidence of diastolic LV dysfunction was therefore 9.4% (CI, 9.1–9.7%). Of 135 participants with diastolic LV dysfunction at baseline, 27 progressed, while 108 maintained their status or regressed (Supplementary Material Table S4). The progression rate was therefore 20.0% (CI, 19.2–20.8%). With adjustment for the baseline covariables, the odds ratios of developing diastolic LV dysfunction or of progressing across stages of diastolic LV dysfunction in relation to glucose + AA taurine were 0.64 (CI, 0.44–0.94; $p = 0.0059$) and 0.63 (CI, 0.46–0.86; $p = 0.0039$), respectively.

PLS analysis

Figure 1 shows the overlaid V-plots for the cross-sectional associations of e' (Figure 1(a)) and E/e' (Figure 1(b)) with the metabolites at baseline (2005–2010) and follow-up (2009–2013) and for the longitudinal associations of e' (Figure 1(c)) and E/e' (Figure 1(d)) at follow-up (2009–2013) with the baseline metabolites (2005–2010). The markers in the top left quadrant (lower e' and higher E/e') and top right quadrant (higher e' and lower E/e') of the V-plots are respectively indicative of worse or better diastolic LV function. Markers with a VIP score consistently higher than 1.1 in the cross-sectional analysis of the baseline and follow-up data and in the longitudinal analysis are listed in Table 3. Markers associated with worse diastolic LV function included glucose + AA glutamine and SCFA pentanoate, whereas metabolites consistently associated with better diastolic LV function comprised AAs 2-aminobutyrate and 4-hydroxybutyrate and BCAAs leucine and valine.

Pathway analysis

In the pathway analysis (Figure 2), we included 27 metabolites (bold in Supplementary Material Table S1), i.e. those with a VIP score higher than 1.1 in any of the four V-plots (Figure 1) and the AA taurine predictive of better diastolic LV function in the longitudinal analysis of single markers. On the basis of human data available in the Kyoto Encyclopaedia of Genes and Genomes (KEGG [https://www.genome.jp/kegg/pathway.html]), the most important pathways ($-\log10_{p\text{-value}} > 5$) included: BCAA metabolism (12.6), aminoacyl-tRNA biosynthesis (9.9), taurine and hypotaurine metabolism (7.1), alanine, aspartate and glutamate metabolism (6.6), glycolysis and gluconeogenesis (5.9), and propanoate metabolism (5.5).

Discussion

Circulating metabolic markers consistently associated with more performant diastolic LV function in the multi-marker analyses were the AAs 2-aminobutyrate and 4-hydroxybutyrate and the BCAAs leucine and valine, while those consistently associated with worse diastolic LV function comprised glucose + AA glutamine and SCFA pentanoate (Table 3 and Figure 1). Single markers associated with more performant diastolic LV function in the cross-sectional analysis at follow-up (Table 2) included valine, glucose + taurine and 2-oxobutyrate, whereas pentanoate was associated with worse function. Mediation analysis suggested that association of diastolic LV function with valine, 2-oxobutyrate and pentanoate were partly direct and partly indirect mediated via LV mass index, whereas the association with glucose + taurine was a direct association only. BCAA metabolism and aminoacyl-tRNA biosynthesis were among the top metabolic pathways associated with diastolic LV dysfunction.

Diastolic relaxation requires high amounts of energy.[18] One of the most studied metabolic signatures of heart failure is the shift from a dominant fatty acid bio-energetic state into a more glycolytic state, viewed as a compensatory response with the purpose of enhancing the utilisation of energy substrate and oxygen.[18] In our current analysis, glucose + AA glutamine and SCFA pentanoate were associated with worse diastolic LV function, which may reflect the balance between anaerobic and aerobic energy generation (Supplementary Material Figure S3). Indeed, glutamine and SCFAs are alternative anaplerotic substrates that

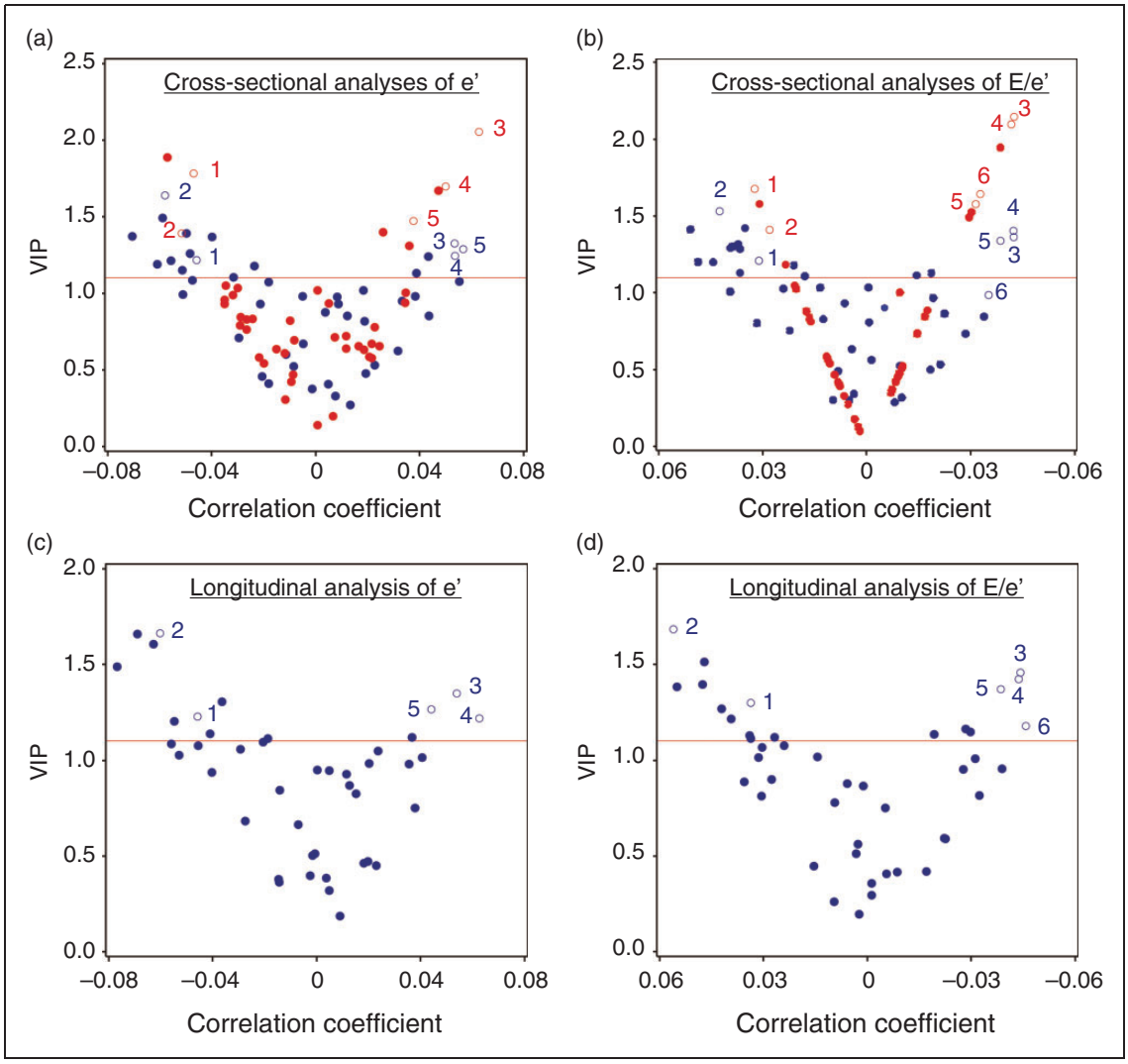

Figure 1. Overlaid V-plots for the cross-sectional associations of e' (a) and E/e' (b) with the metabolites at baseline (2005–2010, blue) and follow-up (2009–2013, red) and for the longitudinal associations of e' (c) and E/e' (d) at follow-up (2009–2013) with the baseline metabolites (2005–2010). The variable importance in projection (VIP) score indicates the importance of a metabolite in the construction of the partial least squares factors and is plotted against the centred and rescaled correlation coefficients, which reflect the associations of the echocardiographic measurements with the metabolites. For E/e' the *x*-axis was inverted to be directionally similarly associated with worse diastolic LV function as for e'. Markers in the top left quadrant (lower e' and higher E/e') and top right quadrant (higher e' and lower E/e') of the V-plots are respectively associated with worse or better diastolic left ventricular function. Markers with a VIP score consistently higher than 1.1 are listed in Table 3. Numbers identify metabolites: (1) pentanoate; (2) glucose + glutamine; (3) 4-hydroxybutyrate; (4) 2-aminobutyrate; (5) leucine; and (6) valine.

can replenish the tricarboxylic acid cycle (TCA) cycle intermediates and thereby generate energy via aerobic oxidation.[19] However, glutamine is also a precursor of proline and hydroxyproline, which are building blocks of collagen.[20] Thus higher glutamine levels may also reflect LV fibrosis and stiffening of the heart, which can explain the association with lower e' (Table 3 and Figure 1).

The molecular mechanisms underlying left ventricular dysfunction recently underwent a paradigm shift moving away from the balance between fatty acid and glucose utilisation as energy sources towards BCAA metabolism as another potentially important contributor.[21] In line with these novel insights,[21] we noticed that valine and leucine (Tables 2 and 3) were associated with better diastolic LV function, while in pathway analysis BCAA metabolism appeared as the top molecular mechanism associated with diastolic LV function (Figure 2). The BCAAs valine, leucine and isoleucine share structural features in the side-chain and have a common catabolic pathway. They are indispensable building blocks in protein synthesis and

Table 3. Parameter estimates in partial least squares analysis.

Tissue Doppler Index metabolite	Cross-sectional analysis of baseline data (2005–2010)		Cross-sectional analysis of follow-up data (2009–2013)		Longitudinal analysis (2005–2010 → 2009–2013)	
	VIP	*r*	VIP	*r*	VIP	*r*
e'						
2-Aminobutyrate	1.24	+0.043	1.67	+0.047	1.26	+0.045
Glucose+glutamine	1.64	–0.058	1.39	–0.052	1.66	–0.060
4-Hydroxybutyrate	1.33	+0.043	2.05	+0.063	1.35	+0.054
Leucine	1.28	+0.048	1.47	+0.038	1.26	+0.045
Pentanoate	1.22	–0.046	1.78	–0.047	1.23	–0.046
E/e'						
2-Aminobutyrate	1.39	–0.042	2.10	–0.042	1.46	–0.044
Glucose+glutamine	1.53	+0.043	1.41	+0.028	1.68	+0.056
4-Hydroxybutyrate	1.37	–0.042	2.15	–0.043	1.42	–0.044
Leucine	1.34	–0.038	1.58	–0.031	1.37	–0.039
Pentanoate	1.21	+0.031	1.68	+0.033	1.30	+0.034
Valine	0.98	–0.038	1.64	–0.033	1.18	–0.046

The variable importance in projection (VIP) score indicates the importance of a metabolite in the construction of the partial least squares factors. The centred and rescaled correlation coefficient, *r*, reflects the associations of the echocardiographic measurements with the metabolites. Listed are the parameter estimates, which were consistent in the cross-sectional analysis of the baseline (2005–2010) and follow-up (2009–2013) data and in the longitudinal analysis relating e' and E/e' at follow-up (2009–2013) to the baseline metabolites (2005–2010). For valine in the cross-sectional analysis of the baseline data, the VIP score was lower than the 1.1 threshold.

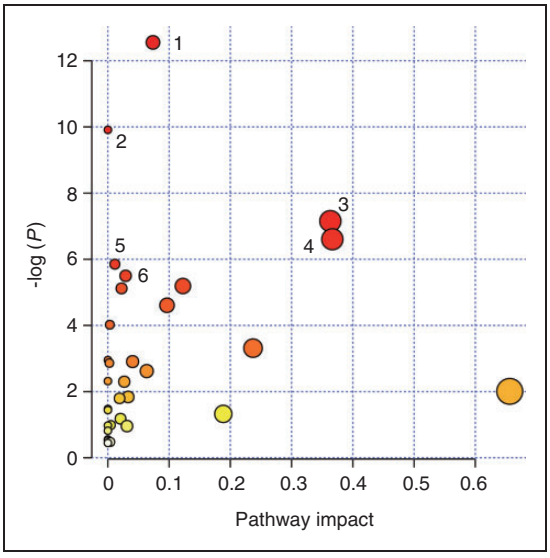

Figure 2. Metabolic pathway analysis based on human data in the Kyoto Encyclopaedia of Genes and Genomes. The analysis included 27 metabolites with a VIP score higher than 1.1 (Figure 1 and Supplementary Material Table S1). The size of the plotted points is proportional to the impact of the pathway. Metabolic pathways of potential relevance ($-\log10_{p\text{-value}}>5$) for the association with diastolic LV function are annotated: (1) BCAA metabolism (12.6); (2) aminoacyl tRNA biosynthesis (9.9); (3) taurine and hypotaurine metabolism (7.2); (4) alanine, aspartate and glutamate metabolism (6.6); (5) glycolysis and gluconeogenesis (5.9); and (6) propanoate metabolism (5.5).

are involved in the aminoacyl-tRNA biosynthesis (Supplementary Material Figure S4). BCAAs are catabolised to branched-chain keto acids (BCKAs) by a branched-chain aminotransferase (Figure 3). BCKAs complexed with the BCKA dehydrogenase require a mitochondrial protein phosphatase (PP2Cm) for further catabolism to acetyl-coenzyme A (CoA), which can enter the TCA cycle.[21] BCAAs are essential nutrients, which implies that their homeostasis is regulated via their catabolic turnover. Unlike other AAs, BCAA catabolism mainly occurs in non-hepatic tissues, particularly also in cardiomyocytes.[22] Due to their poor hepatic catabolism, circulating BCAAs act as nutritional sensors and in this role interact with mammalian target of rapamycin (mTOR), thereby uncoupling insulin signalling (Supplementary Material Figure S3).[23]

Recent experimental studies[24–27] support the implication of BCAAs in modulating LV function. In a salt-loaded rat model of heart failure[24] and in middle-aged mice,[25] BCAA dietary supplements promoted cardiomyocyte survival,[24] preserved cardiac function[25] and prolonged lifespan.[24,25] In the failing heart of PP2Cm knock-out mice, myocardial accumulation of BCKAs was a prominent feature, which was subsequently replicated in a human case-control study of dilated cardiomyopathy.[26] Expression of PP2Cm in the heart is dynamically regulated by stressors. In hypertrophic and failing hearts, its expression is significantly reduced at both the mRNA and protein level.[27] Elevated

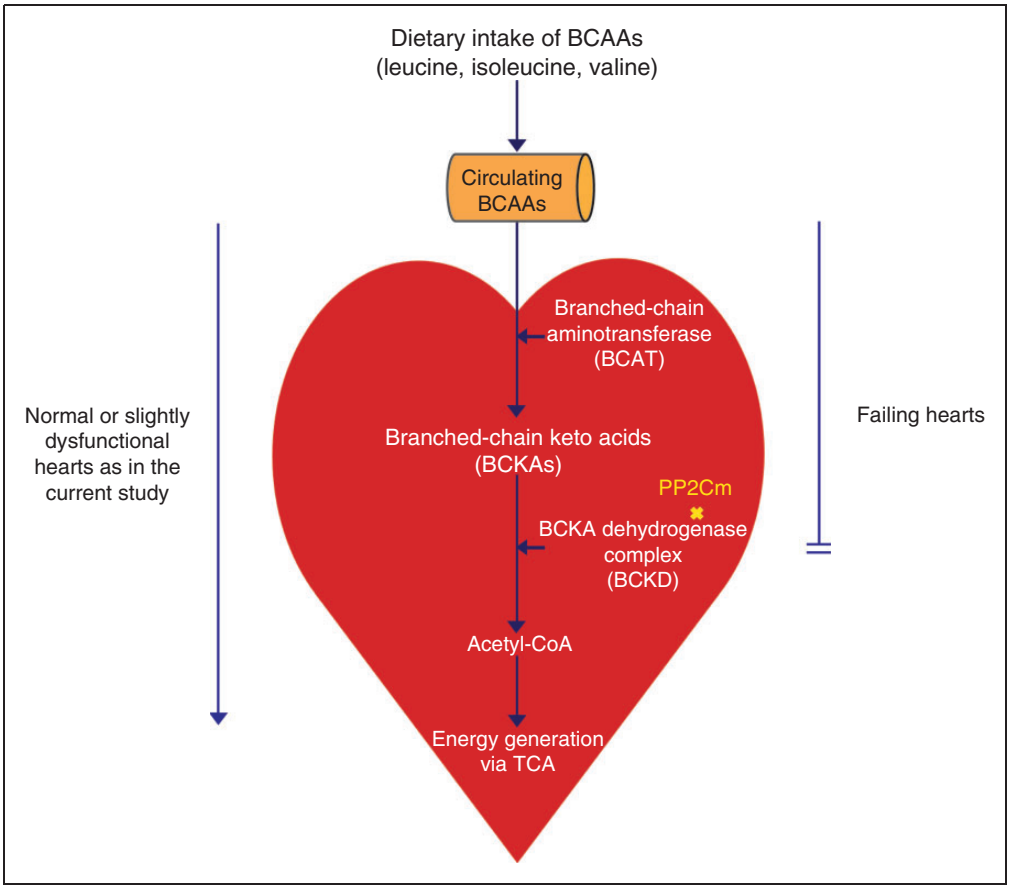

Figure 3. Take home figure. Differential role of the branched-chain amino acid catabolic pathway in normal and failing hearts – a hypothesis supported by the current data and the literature (see discussion). The branched amino-acids (BCAAs) leucine, isoleucine and valine are essential nutrients. The first step of BCAA catabolism mainly takes place in non-hepatic tissues, including cardiomyocytes. BCAAs are catabolised to branched-chain keto acids (BCKAs) by a branched-chain aminotransferase (BCAT). BCKAs complexed with the BCKA dehydrogenase require a mitochondrial protein phosphatase (PP2Cm) for further catabolism to acetyl-coenzyme A (CoA), which can enter the tricarboxylic acid cycle (TCA) and contribute to energy generation. Circulating BCAA levels, reflecting the balance between nutritional intake and catabolism, may be beneficial in normal or slightly dysfunctional hearts. Expression of mitochondrial proteins, including PP2Cm, is reduced in failing hearts. BCAAs become harmful in advanced heart failure when their catabolism is defective and cardiotoxic BCKAs accumulate in the myocardium.

circulating BCAA levels reflect a healthy nutritional state and must be beneficial in normal or slightly dysfunctional hearts, but might become harmful when BCAA catabolism is defective and cardiotoxic BCKAs accumulate in the failing myocardium (Figure 3).

Metabolic markers associated with more performant diastolic LV function also included glucose + taurine and 2-aminobutyrate (Tables 2 and 3; Figure 1). The AA taurine inhibits the generation of reactive oxygen species and taurine administration to heart failure patients improves end-diastolic LV volume, symptoms, and exercise capacity.[28] The AA 2-aminobutyrate reflects hepatic glutathione (GSH) consumption.[29] The anti-oxidant GSH provides systemic protection against oxidative stress. In the presence of low hepatic availability of cysteine, the rate-limiting precursor in GSH biosynthesis, 2-aminobutyrate enters an alternative pathway (Supplementary Material Figure S3) driven by the same enzymes leading to the synthesis of ophthalmate.[29] Higher circulating levels of 2-aminobutyrate therefore reflect less hepatic uptake of this AA and lower systemic oxidative stress. This might explain the association of a more performant diastolic LV function with higher serum 2-aminobutyrate. In keeping with this hypothesis, oral administration of 2-aminobutyrate in a mouse model of cardiomyopathy induced by oxidative stress efficiently raised both circulating and myocardial GSH levels.[30]

Strong points of our current study are the consistency in the identification of the metabolic markers over time in a sample of people representative of the general population and of the initial transition from normal LV function to asymptomatic diastolic LV dysfunction.

Moreover, our current findings are in line with recent research developments focusing on the role of a defective BCAA catabolism in the pathogenesis of LV dysfunction.[24–27] As in the Framingham Heart Study,[7] we adjusted significance levels for multiple testing by clustering the metabolites into six groups. PLS analysis allowed entering 43 highly inter-correlated markers together into a single computer run without the need to adjust the significance levels. Nevertheless, our study must also be interpreted within the context of its potential limitations. First, this report is the first population study relating diastolic LV function to circulating BCAAs, but does not include a replication in a different cohort. However, we reproduced our main observations at a five-year interval. Although the sample size is relatively small, our study satisfies the Bradford-Hill criteria,[31] of consistency (between baseline and follow-up), temporality (baseline markers predicting diastolic LV dysfunction at follow-up), plausibility (Supplementary Material Figure S3) and coherence (between clinical and experimental observations).[24–27] Second, the attrition rate between baseline and follow-up was 19.8%. However, participants dropping out had broadly similar characteristics as those remaining in follow-up (Supplementary Material Table S2). Third, metabolomics performed on circulating blood provide a snapshot of the metabolic composition of a sample. Keeping in mind the redundancy and bidirectionality in many metabolic pathways,[32] without additional measurements in multiple serial samples, studies like ours cannot determine whether levels of circulating metabolites vary because of changes in production or degradation or release into or clearance from the circulation, or reflect a combination of these mechanisms. However, it is possible to determine the metabolic cores involved and postulate explicative mechanisms based on the combination of metabolomics data and other physiological/clinical information. Fourth, NMR spectroscopy is fast and keeps samples separated from the instrument, but produces crowded spectra that cannot always be reliably deconvoluted to single metabolites.[33] However, differently to other metabolomics platforms, NMR is very reproducible and sensitive to quantitative (vs qualitative) changes in metabolic composition. Finally, our findings are representative for people with normal or slightly impaired diastolic LV function with normal LV ejection fraction and can therefore not be extrapolated to systolic LV dysfunction or advanced heart failure.

Conclusions

In previous publications, we described the association of diastolic LV function with genetic variation in candidate genes,[34] circulating markers of collagen turnover [35] or cardiomyocyte injury,[36] multidimensional urinary proteomic classifiers,[37,38] and parental proteins identified from sequenced urinary peptides.[35] In general, genetics and epigenetics are far from the phenotype, proteomics occupy a middle position and metabolomics are close. However, circulating metabolic markers are typically considered to be volatile at best and to represent only a snapshot of an underlying pathologic process. We showed that among people covering the range from normal to asymptomatic diastolic LV dysfunction, there was consistency over five years in the associations of diastolic LV function with metabolic markers. Demonstrating temporal consistency in the associations between a phenotype and a biomarker is key for the clinical application of biomarkers. In addition to disturbances in the balance between aerobic and anaerobic energy generation, our observations suggested a key role of BCAA metabolism and aminoacyl-tRNA biosynthesis in maintaining diastolic LV performance. These observations open new research perspectives for treatment and early prevention of heart failure, for instance by pharmacologically activating the defective mitochondrial phosphatase PP2Cm in the failing myocardium or by dietary BCAA supplementation to non-diabetic[23] patients at risk for heart failure.

Author contribution

Z-ZY and JAS conceived and designed the study and drafted the report, with key input from DM. Z-ZY, LT, and JAS did the statistical analyses. VGM, DM, and JR measured serum metabolites. W-YY, J-UV, and TK supported the echocardiographic studies. ST, Q-FH, F-FW, and JVK played key roles in recruitment of participants, or generation of data. DM, JR, and JAS obtained funding. All authors provided critical input into revised versions of the report.

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Declaration of conflicting interests

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References

1. Ussher JR, Elmariah S, Gerszten RE, et al. The emerging role of metabolomics in the diagnosis and prognosis of cardiovascular disease. *J Am Coll Cardiol* 2016; 68: 2850–2870.
2. GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: A systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; 388: 1545–1602.
3. Dunn WB, Broadhurst DI, Deepak SM, et al. Serum metabolomics reveals many novel metabolic markers of heart failure, including pseudouridine and 2-oxoglutarate. *Metabolomics* 2007; 3: 413–426.
4. Cheng ML, Wang CH, Shiao MS, et al. Metabolic disturbances identified in plasma are associated with outcomes in patients with heart failure: Diagnostic and prognostic value of metabolomics. *J Am Coll Cardiol* 2015; 65: 1509–1520.
5. Hunter WG, Kelly JP, McGarrah RW3, et al. Metabolomic profiling identifies novel circulating biomarkers of mitochondrial dysfunction differentially elevated in heart failure with preserved versus reduced ejection fraction: Evidence for shared metabolic impairments in clinical heart failure. *J Am Heart Assoc* 2016; 5: e003190.
6. Ko D, Riles EM, Marcos EG, et al. Metabolomic profiling in relation to new-onset atrial fibrillation (from the Framingham Heart Study). *Am J Cardiol* 2016; 118: 1493–1496.
7. Cheng S, Larson MG, McCabe EL, et al. Distinct metabolomic signatures are associated with longevity in humans. *Nat Commun* 2015; 6: 6791.
8. Kuznetsova T, Herbots L, López B, et al. Prevalence of left ventricular diastolic dysfunction in a general population. *Circ Heart Fail* 2009; 2: 105–112.
9. Kloch-Badelek M, Kuznetsova T, Sakiewicz W, et al., on behalf of the European Project on Genes in Hypertension (EPOGH) Investigators. Prevalence of diastolic left ventricular dysfunction in European populations based on cross-validated diagnostic thresholds. *Cardiovascular Ultrasound* 2012; 10: 10.
10. Kuznetsova T, Thijs L, Knez J, et al. Longitudinal changes in left ventricular diastolic dysfunction in a general population. *Circ Cardiovasc Imaging* 2015; 8: e002882.
11. Kuznetsova T, Thijs L, Knez J, et al. Prognostic value of left ventricular diastolic dysfunction in a general population. *J Am Heart Assoc* 2014; 3: e000789.
12. Zhang ZY, Marrachelli VG, Thijs L, et al. Diastolic left ventricular function in relation to circulating metabolic biomarkers in a general population. *J Am Heart Assoc* 2016; 5: e002681.
13. Levey AS, Stevens LA, Schmid CH, et al., for the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; 150: 604–612.
14. Gottdiener JS, Bednarz J, Devereux R, et al. American Society of Echocardiography recommendations for use of echocardiography in clinical trials. A report from the American Society of Echocardiography's Guidelines and Standard Committee and the Task Force on Echocardiography in Clinical Trials. *J Am Soc Echocardiogr* 2004; 17: 1086–1119.
15. Marrachelli VG, Monleon D, Rentero P, et al. Genomic and metabolomic profile associated to microalbuminuria. *PLoS One* 2014; 9: e98227.
16. Dona AC, Coffey S and Figtree G. Translational and emerging clinical applications of metabolomics in cardiovascular disease diagnosis and treatment. *Eur J Prev Cardiol* 2016; 23: 1578–1589.
17. Kunutsor SK, Laukkanen JA, Bluemke DA, et al. Baseline and long-term gamma-glutamyltransferase, heart failure and cardiac arrhythmias in middle-aged Finnish men: Prospective study and pooled analysis of published evidence. *Eur J Prev Cardiol* 2016; 23: 1354–1362.
18. Brown DA, Perry JB, Allen ME, et al. Mitochondrial function as a therapeutic target in heart failure. *Nature Rev Cardiol* 2017; 14: 238–250.
19. Reitzer LJ, Wice BM and Kennell D. Evidence that glutamine, not sugar, is the major energy source for cultured HeLa cells. *J Biol Chem* 1979; 254: 2669–2676.
20. Phang JM, Liu W, Hancock CN, et al. Proline metabolism and cancer: Emerging links to glutamine and collagen. *Curr Opin Clin Nutr Metab Care* 2015; 18: 71–77.
21. Huang Y, Zhou M, Su H, et al. Branched-chain amino acid metabolism in heart disease: an epiphenomenon or a real culprit? *Cardiovasc Res* 2011; 90: 220–223.
22. Harper AE, Miller RH and Block KP. Branched-chain amino acid metabolism. *Annu Rev Nutr* 1984; 4: 409–454.
23. Newgard CB, An J, Bain JR, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab* 2009; 9: 311–326.
24. Tanada Y, Shioi T, Kato T, et al. Branched-chain amino acids ameliorate heart failure with cardiac cachexia in rats. *Life Sci* 2015; 137: 20–27.
25. D'Antona G, Ragni M, Cardile A, et al. Branched-chain amino acid supplementation promotes survival and supports cardiac and skeletal muscle mitochondrial biogenesis in middle-aged mice. *Cell Metab* 2010; 12: 362–372.
26. Sun H, Olson KC, Gao C, et al. Catabolic defect of branched-chain amino acids promotes heart failure. *Circulation* 2016; 133: 2038–2049.
27. Lu G, Sun H, She P, et al. Protein phosphatase 2 Cm is a critical regulator of branched-chain amino acid catabolism in mice and cultured cells. *J Clin Invest* 2009; 119: 1678–1687.
28. Beyranvand MR, Khalafi MK, Roshan VD, et al. Effect of taurine supplementation on exercise capacity of patients with heart failure. *J Cardiol* 2011; 57: 333–337.
29. Dello SA, Neis EP, de Jong MC, et al. Systematic review of ophthalmate as a novel biomarker of hepatic glutathione depletion. *Clin Nutr* 2013; 32: 325–330.
30. Irino Y, Toh R, Nagao M, et al. 2-Aminobutyric acid modulates glutathione homeostasis in the myocardium. *Sci Rep* 2016; 9: 36749.

31. Fedak KM, Bernal A, Capashaw ZA, et al. Applying the Bradford Hill criteria in the 21st century: How data integration has changed causal inference in molecular epidemiology. *Emerg Themes Epidemiol* 2015; 12: 14.
32. Czibik G, Steeples V, Yavari A, et al. Citric acid cycle intermediates in cardioprotection. *Circ Cardiovasc Genet* 2014; 7: 711–719.
33. Griffin JL, Atherton H, Shockcor J, et al. Metabolomics as a tool for cardiac research. *Nat Rev Cardiol* 2011; 8: 630–643.
34. Knez J, Salvi E, Tikhonoff V, et al. Left ventricular diastolic function associated with common genetic variation in ATP12A in a general population. *BMC Med Genet* 2014; 15: 121.
35. Zhang ZY, Ravassa S, Yang WY, et al. Diastolic left ventricular function in relation to urinary and serum collagen biomarkers in a general population. *PLoS One* 2016; 11: e0167582.
36. Ravassa S, Kuznetsova T, Varo N, et al. Biomarkers of cardiomyocyte injury and stress identify left atria and left ventricular remodelling and dysfunction: A population-based study. *Int J Cardiol* 2015; 185: 177–185.
37. Zhang ZY, Staessen JA, Thijs L, et al. Left ventricular diastolic function in relation to the urinary proteome: A proof-of-concept study in a general population. *Int J Cardiol* 2014; 176: 158–165.
38. Zhang ZY, Nkuipou-Kenfack E, Yang WY, et al. Epidemiologic observations guiding clinical application of the HF1 urinary peptidomic marker of diastolic left ventricular dysfunction. *J Am Soc Hypertens* 2018; 12: 438–447.