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Metabolomic Distinction and Insights Into the Pathogenesis of Human Primary Dilated Cardiomyopathy

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Abstract

Background—Metabolomics, the comprehensive profile of small molecule metabolites found in biological specimens, has the potential to provide insights into the pathogenesis of disease states and lead to identification of new biomarkers.

Methods and Results—We analyzed 451 plasma metabolites by liquid chromatography/mass spectroscopy and gas chromatography/mass spectroscopy in 39 patients with primary dilated cardiomyopathy (DCM) and 31 age-, sex- and body mass index-matched controls. Sixty-one metabolites were significantly different between primary DCM and control individuals (FDR < 0.05). Plasma levels of steroid metabolites, glutamine, threonine and histidine were reduced while levels of citric acid cycle intermediates and lipid β-oxidation products were increased in patients with primary DCM as compared to controls. Medications, particularly furosemide and angiotensin-1 converting enzyme-1 inhibitors, had significant effects on the plasma metabolites. Reduced levels of glutamine in conjunction with increased 3-methyhistidine and prolylhydroxyproline levels suggested enhanced myofibrillar and collagen degradation in DCM patients. Likewise, increased stachydrine and reduced indole-3-propionate implicated a role for intestinal derived anti-oxidant molecules. Changes in steroid metabolites were notable for the loss of metabolic distinction between males and females in patients with primary DCM. Cortisol and cortisone levels were increased while androgen metabolites were decreased significantly, implying metabolic “feminization” of primary DCM males.

Conclusions—Metabolomic profiling identifies biologically active metabolites that could serve as markers of primary DCM and impart protective or harmful effects on cardiac structure and function.

Keywords

Heart Failure; Cardiomyopathy; Metabolites

Heart failure affects approximately 23 million individuals worldwide and more than five million Americans [1]. Heart failure is responsible for over one million hospitalizations and over a quarter of million deaths per year [1]. It is the only cardiovascular disease for which the incidence continues to increase [1]. During the past two decades, there have been considerable advances in the treatment of systolic heart failure. The advents of angiotensin-1

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DISCLOSURE Danny Alexander and Matthew Mitchells are employees of Metabolon, Inc.

converting enzyme-1 (ACE-1) inhibitors and β blockers have reduced mortality by 20% and 30%, respectively [2-4]. Despite the advances, mortality and morbidity of patients with heart failure remain high and comparable to many cancers [1].

Primary dilated cardiomyopathy (DCM) is the prototypic form of systolic heart failure, which is characterized by cardiac dilatation in conjunction with reduced left ventricular ejection fraction (LVEF). Primary DCM, often caused by mutations in cytoskeletal and sarcomeric proteins [5]. DCM also represents common phenotypic responses of the heart to various form of injury or stress, namely, cardiac dilatation and dysfunction.

The metabolome has been defined as the complete profile of small molecule metabolites found in a specific cell, organ or organism [6]. Metabolomic analysis has the potential to provide insights into metabolic pathways in specific cells, organs or organisms [7]. Metabolomics is the high-throughput identification, quantification and characterization of the small molecule metabolites simultaneously [6]. The metabolome could represent the cumulative end product of gene expression, environmental factors and the complex multi-faceted interactions between them. It has the potential to lead to elucidation of molecular pathways involved in the pathogenesis of the phenotype and hence, identification of novel diagnostic markers and therapeutic targets. In the present study, we analyzed the plasma metabolites in patients with primary DCM and normal individuals.

METHODS

Study population

The Institutional Review Board approved the study and the participants gave informed consent. The study population included 70 individuals, comprising 39 cases with primary DCM and 31 normal individuals (controls). Primary DCM was diagnosed based on the conventional criteria of a LVEF of less than 45%, a dilated left ventricle (an end diastolic diameter of greater than 2.7 cm/m2) and no known causes of myocardial disease [8]. Accordingly, various secondary causes of heart failure including coronary artery disease and valvular diseases were excluded in order to diminish the potential confounding effects of etiological heterogeneity of heart failure. A control group of normal individuals, defined as asymptomatic age- and sex- matched individuals with normal electrocardiogram and echocardiograms was included. A normal echocardiogram excludes the diagnosis of DCM in the control individuals. To reduce the potential confounding effects of race and diabetes mellitus, the study was conducted in the Caucasians only group and those with diabetes mellitus were excluded.

Sample accessioning

Each sample was accessioned into the Metabolon, Inc. (Research Triangle, NC) Laboratory Information Management System (LIMS). Each sample was assigned by the LIMS a unique identifier. All samples were maintained at −80 °C until processed.

Metabolomic analysis

Detailed procedure for sample extraction and metabolomic analysis is described in a recent technical manuscript [9]. Briefly, the extracted sample was split for analysis on three analytical platforms, Gas Chromatography/Mass Spectroscopy (GC/MS), Ultra High Performance Liquid Chromatography (UHPLC)/MS-Positive, and UHPLC/MS-Negative. Following peak identification and quality control filtering, integrated peak ion counts for each compound in each sample were used for statistical analysis.

Statistical analysis

We analyzed the distributions of the baseline phenotypic characteristics of the study populations by Shapiro-Wilk normality test. We expressed those that followed the Gaussian distribution as mean ± SD. We compared the mean values between the two groups by Student's t test and analyzed the non-parametric variables by Fisher exact test. The parametric variables that deviated from normality distribution were analyzed by Kuskal-Wallis test. For this set of analysis we used STATA 10.1 software for Macintosh.

The statistical analysis program "R" was used for analysis of metabolites (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria URL:http://www.R-project.org). Whenever a compound level was below the level of detection in some samples (missing values) and not others, we provided an imputed value equal to the minimum observed among all the samples of the study (including DCM and controls). While this may lead to an artificially high mean in a group with multiple imputed values, it is a conservative substitution, which underestimates the fold-change between the test and control groups. Analysts take this into account when interpreting data in the context of metabolic pathways.

The mean values for the metabolites were determined based on a table of median scaled data for each compound; *i.e.* each data point is divided by the median of all detected values for that compound on that day. Day-block normalization requires balanced numbers of case and control samples in each day set. To accomplish a balanced number, samples were coded such that sets of approximately 30 samples (one day's run) contain equal numbers of cases and controls. These data sets are provided in a blinded fashion to the analytical laboratory. The day-block sets are then randomized for analysis within each day. This procedure was done to remove any day-to-day instrument drift, and as a result, each data point is represented as a fraction of the median value for that day. The fold-changes represent ratios of the means.

Welch's two-sample t-test was performed on the log-transformed data. To account for multiple comparisons, we used the false discovery rate (FDR) method [10], and we estimated the q-value using the method of Storey and Tibshirani [11], using a cut-off point for statistical significance of <0.05 (*i.e.* FDR < 5%).

RESULTS

Baseline characteristics of the study populations

Baseline characteristics of the study populations including their phenotypic data are presented in Table 1. The groups were matched for age, sex, height, weight and body mass index (BMI). Per inclusion criteria, all participants were Caucasians and there was no individual with diabetes mellitus. Primary DCM patients and controls differed in several baseline characteristics including systolic blood pressure (lower in those with DCM) and the New York Heart Association (NYHA) functional class (higher in DCM). In addition, one third of patients with primary DCM were in normal sinus rhythm, as opposed to 100% of controls. There were no smokers in primary DCM group, as compared to 10% in the control group. As anticipated, patients with primary DCM were on treatment with β blockers, inhibitors of the renin-angiotensin-aldosterone system (RAAS) and diuretics (Table 1). Finally, as per study design, the echocardiographic indices of cardiac dilatation and function were significantly different between the cases and controls.

Compound summary and process evaluation

451 compounds passed criteria for analytical quality control (QC), data curation, and curation QC (Online supplementary material-Table 1). 281 compounds matched a purified standard compound entry in the Metabolon@ reference library [9](Online supplementary material-Table 1) and are thus referred to as "named" compounds.. The remainder matched parent ion and fragmentation ion spectra observed recurrently in Metabolon@ plasma projects, and thus represent unnamed compounds rather than merely observable individual ions.

Analytical process control was viewed at two levels; 1) variation in the instrument process only, and 2) variation in the overall process (Online supplementary material Table 2). The median relative standard deviation (RSD = 100×CV) for measuring 30 internal standard compounds was 6.5% (variation in instrument process only). The median RSD of combined variation of all processes, including extraction, derivatization, recovery, and the instrument process for 280 compounds which were detected in a series of 24 technical replicate plasma samples embedded within the sample runs (MTRX samples), was 14.4%.

In contrast to the MTRX, the median RSD for 346 compounds detected in 80% or more of the control group study samples was 36% (*i.e.* process plus background biological variation), while the median RSD over all the study groups was 51.5%. The findings indicate a large phenotype contribution to the data.

Metabolomic distinction of primary DCM patients

Plasma levels of 61 named compounds were changed significantly in primary DCM patients, as compared to controls ($q<0.05$). Plasma levels of 41 named compounds were increased, including methylglutaroylcarnitine, taurocholenate sulfate, 4-acetamidobutanoate, alpha-ketoglutarate, pro-hydroxy-pro and cortisol, which were increased by ~ 2- to 3- fold (Table 2). In contrast, plasma levels of 20 metabolites were decreased significantly (Table 3). Among the notable reductions were plasma levels of 5α-pregnan-3 β, 20α-diol disulfate, iminodiacetate (IDA), deoxycholate, paraxanthine, and dehydroisoandrosterone sulfate (DHEA-S), which were reduced by about 50% or more (Table 3).

Sex-dependent changes in plasma metabolites in DCM patients

There were significant differences in the plasma metabolites between male and female individuals in the control group (Table 4). For example, plasma levels of 4-androsten-3β, 17β-diol monosulfate 1, 4-androsten-3β,17β-diol disulfate 1, DHEA-S, 4-androsten-3β,17β-diol disulfate 2, Pregn steroid monosulfate, 5α-androstan-3β,17β-diol disulfate and Epiandrosterone sulfate were significantly different between males and females in the control group (Table 4). However, individuals with primary DCM exhibited no significant differences between males and females for these plasma metabolites (at $q<0.05$). That is, comparing the same plasma metabolites separately in females and males, no significant differences were detected between control and primary DCM groups for females, but the metabolites were significantly different between the control and primary DCM groups for males (Table 5). In male individuals with primary DCM, plasma metabolites of sterol/steroid pathway 4-androsten-3β,17β-diol monosulfate 1, DHEA-S, Pregn steroid monosulfate, Epiandrosterone sulfate, 1-eicosatrienoylglycerophosphocholine were significantly reduced as compared to controls. By contrast, Choline, Acetylcarnitine, Isobutyrylcarnitine, Methylglutaroylcarnitine, γ-glutamylisoleucine, γ-glutamyltyrosine, Dimethylarginine (SDMA + ADMA) and several others were increased significantly in male individuals with primary DCM (Table 5).

Effects of medications

The majority of the individuals with primary DCM were taking β blockers, ACE inhibitors and/or furosemide (Table 1). To determine the impacts of the medications on plasma metabolites, we analyzed differences in the plasma metabolites in individuals not taking a specific drug and controls. As would be expected, removal of subjects treated with each class of medications decreased the number of metabolites that were significantly altered in primary DCM group, partly because of the effects of the medications and in part because of reduced sample size (reduced power). Treatment with β blockers had modest effects on plasma levels of metabolites. Plasma levels of 23 metabolites were significantly different between control individuals and 7 individuals with primary DCM who were not treated with β blockers (Table 6). Treatment with ACE inhibitors and furosemide had the strongest effects. Plasma level of only 1-linoleoylglycerophosphocholine was significantly reduced (~ 40%) in the cases with primary DCM not treated with ACE inhibitors (N=13) as compared to controls (N=31). Likewise, only plasma glutamine level was significantly reduced in the primary DCM individuals not treated with furosemide (N=13) as compared to controls (N=31). The complete results are shown in Table 6.

DISCUSSION

We analyzed plasma metabolites in Caucasian patients with primary DCM and detected significant differences between primary DCM patients and the control individuals. Metabolomic profile of primary DCM patients was characterized by decreased levels of steroid metabolites, glutamine, threonine and histidine and increased levels of citric acid cycle intermediates and lipid β-oxidation products. In addition, the sex-dependent differences in the plasma metabolites were largely absent in the primary DCM group. Notably, females in the control and primary DCM groups had a similar plasma metabolite profile, while, androgen metabolites, such as DHEA-S and epiandrosterone sulfate were significantly reduced in male patients with primary DCM, as compared to controls. Consequently, metabolic profile of male patients with primary DCM, as regards the androgenic metabolites, largely resembled the profile in normal females. These findings imply metabolic “feminization” of male patients with primary DCM.

Metabolism, as reflected in the metaoblome, is a dynamic process subject to the influence of numerous intrinsic and external factors, such as food intake and medications. The measurement of plasma metabolites, while practical, may only partially reflect changes in the myocardial metabolites in primary DCM. The differences in the plasma metabolites, nevertheless, strongly differentiated primary DCM patients from the controls individuals in various metabolic pathways. Plasma levels of several amino acids were reduced significantly, including glutamine, which is the most abundant amino acid in the body [12]. By contrast, plasma level of glutamate, which is converted to glutamine by the cytosolic enzyme glutamine synthetase was increased. The decrease in the glutamate/glutamine ratio may reflect suppression of glutamine synthetase enzymatic activity or the overwhelming of the capacity to produce glutamine by catabolic stress in patients with primary DCM. The biological significance of the changes in glutamate/glutamine is noteworthy, as glutamine is a key regulator of protein homeostasis and inhibits protein degradation [13]. Depletion of glutamine through inhibition of glutamine synthetase markedly reduces protein synthesis in cultured cells [14], whereas, replenishment of glutamine improves nitrogen balance during metabolic stress and limits amino acid oxidation [15]. In accord with the pivotal role of glutamine in protein homeostasis, plasma level of 3-methylhistidine, which is an index of myofibrillar protein degradation [16], was increased significantly in DCM patients. Likewise, plasma level of dipeptide prolylhydroxyproline, a marker of collagen degradation, was also increased. The changes likely reflect increased myofibrillar and collagen proteins degradation in the heart and may contribute to sarcopenia and impaired extracellular matrix

(ECM) integrity in primary DCM. In addition, the changes may reflect metabolic abnormalities in skeletal muscles in patients with primary DCM, either due to shared underling mechanisms and/or secondary skeletal myopathy because of heart failure [17]. In view of these findings, one may postulate that glutamine deficiency contributes to degradation of myofibrillar and ECM proteins in patients with primary DCM and that glutamine replenishment could reverse protein degradation and influence the clinical course of heart failure.

Changes in the levels of plasma metabolites not only may reflect the molecular phenotype in metabolically active tissues but the metabolites could also impart significant biological effects on organ function. Notable among the active metabolites is indole-3-propionate (IPA), a scavenger of hydroxyl radicals, which was decreased by 40%. IPA is known to reduce cellular oxidative stress and death [18]. Hence, reduced level of IPA in DCM would be expected to increase the susceptibility of the myocardium to oxidative stress. In contrast, stachydrine or proline betaine, a phytochemical, was increased significantly in primary DCM patients. Stachydrine is a free radical scavenger that exerts protective effects in the ischemic myocardium [19]. The fascinating aspect of the findings of reduced plasma IPA in primary DCM patients is that IPA is a deamination product of tryptophan formed by symbiotic bacteria in the gastrointestinal tract. The finding suggest that in primary DCM production of IPA by the gastrointestinal bacteria is reduced, probably due to changes in the gastrointestinal microbial flora, either consequent to mucosal congestion or because of medications. The finding implicates a potential role of the gastrointestinal tract in heart failure. In accord with this notion, plasma level of α-ketoglutarate, which is produced from glutamine in the intestine, was increased and glutamine level was reduced in patients with primary DCM. The intestine utilizes glutamine as the main energy fuel in the fasting state and converts glutamine to ammonia and α-ketoglutarate via the action of intestinal glutaminase and glutamate dehydrogenase. Thus, one may speculate that intestine, through reduced generation of IPA, a cardioprotective agent, and increased utilization of glutamine, a regulator of protein homeostasis, contributes to the pathogenesis and/or progression of heart failure. This notion is in accord with the increasing recognition of the role of glut flora not only in heart failure but also in other pathological conditions [20,21].

The majority of the primary DCM patients were taking multiple medications. Many, but not all, of the apparent metabolomic differences observed in the entire study group may be the result of treatment with heart failure medications. Among the commonly used medications, β-blockers had a minor contribution to plasma metabolome, while ACE inhibitors and furosemide had the most drastic contribution. Because of the small number of primary DCM patients who were not on a specific medication, the results in those not being treated are subject to the possibility of type II statistical error (β error). Nevertheless, glutamine was significant decreased even in those not treated with furosemide and the general trend ($q = 0.05$ to 0.10) remained when the analysis was performed on those not treated with medications, despite a loss of statistical significance in some cases. Overall, a number of metabolites were still significantly different between primary DCM patients and the control individuals, after exclusion of those on specific medications.

The study has several limitations. Despite our criteria for the selection of the cases and the inclusion of those without known secondary causes of cardiomyopathy, primary DCM is a genetically heterogeneous disease. The causal mutations/genes in the majority of our cases are not known but likely to encompass rare or “private” mutations in several genes. Thus, genetic heterogeneity of the cases is a potential confounder that could influence the plasma metabolome. Likewise, the use of the medications compounds the ability to discern the direct effects of primary DCM on plasma metabolites. Moreover, there were no specific controls for the content of the diet or physical activities of the participants. The study is also

not designed to determine the potential effects of plasma metabolites on early identification of primary DCM patients, prognostication or individualization of their therapy. Additionally, we did not have access to cardiac tissue to correlate the changes in plasma metabolites with those in the myocardium.

Metabolomic profiling is an emerging field that has the potential to provide significant insight into cardiac metabolism under different pathological conditions [22-24]. Our findings show significant changes in plasma metabolites in patients with primary DCM, including changes in several metabolites with known active biological effects on the myocardium. The findings merit replication and studies to delineate the utility of plasma metabolites in early disease detection, prognostication and treatment of patients with heart failure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

1. Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB, Flegal K, et al. Heart disease and stroke statistics--2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation. 2009; 119:480–6. [PubMed: 19171871]
2. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. The SOLVD Investigators. N Engl J Med. 1991; 325:293–302. [PubMed: 2057034]
3. Packer M, Fowler MB, Roecker EB, Coats AJS, Katus HA, Krum H, et al. Effect of Carvedilol on the Morbidity of Patients With Severe Chronic Heart Failure: Results of the Carvedilol Prospective Randomized Cumulative Survival (COPERNICUS) Study. Circulation. 2002; 106:2194–9. [PubMed: 12390947]
4. Effectiveness of spironolactone added to an angiotensin-converting enzyme inhibitor and a loop diuretic for severe chronic congestive heart failure (the Randomized Aldactone Evaluation Study [RALES]). Am.J.Cardiol. 1996; 78:902–7. [PubMed: 8888663]
5. Chang AN, Potter JD. Sarcomeric protein mutations in dilated cardiomyopathy. Heart Fail Rev. 2005; 10:225–35. [PubMed: 16416045]
6. Wishart DS, Tzur D, Knox C, Eisner R, Guo AC, Young N, et al. HMDB: the Human Metabolome Database. Nucleic Acids Research. 2007; 35:D521–D6. [PubMed: 17202168]
7. Balaban RS. Maintenance of the metabolic homeostasis of the heart: developing a systems analysis approach. Ann N Y Acad Sci. 2006; 1080:140–53. [PubMed: 17132781]
8. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, et al. Contemporary Definitions and Classification of the Cardiomyopathies: An American Heart Association Scientific Statement From the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. Circulation. 2006; 113:1807–16. [PubMed: 16567565]
9. Evans AM, Dehaven CD, Barrett T, Mitchell M, Milgram E. Integrated, Nontargeted Ultrahigh Performance Liquid Chromatography/Electrospray Ionization Tandem Mass Spectrometry Platform

for the Identification and Relative Quantification of the Small-Molecule Complement of Biological Systems. Anal Chem. 2009

10. Benjamin EJH, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society, Series B. 1995; 75:289–300.
11. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc.Natl.Acad.Sci U.S.A. 2003; 100:9440–5. [PubMed: 12883005]
12. Bergstrom J, Furst P, Noree LO, Vinnars E. Intracellular free amino acid concentration in human muscle tissue. J Appl Physiol. 1974; 36:693–7. [PubMed: 4829908]
13. Hankard RG, Haymond MW, Darmaun D. Effect of glutamine on leucine metabolism in humans. Am J Physiol. 1996; 271:E748–54. [PubMed: 8897864]
14. Le Bacquer O, Nazih H, Blottiere H, Meynial-Denis D, Laboisse C, Darmaun D. Effects of glutamine deprivation on protein synthesis in a model of human enterocytes in culture. Am J Physiol Gastrointest Liver Physiol. 2001; 281:G1340–7. [PubMed: 11705738]
15. Wernerman J, Hammarqvist F, Vinnars E. Alpha-ketoglutarate and postoperative muscle catabolism. Lancet. 1990; 335:701–3. [PubMed: 1969067]
16. Morrison WL, Gibson JN, Rennie MJ. Skeletal muscle and whole body protein turnover in cardiac cachexia: influence of branched-chain amino acid administration. Eur J Clin Invest. 1988; 18:648–54. [PubMed: 3147192]
17. Miller MS, Vanburen P, Lewinter MM, Lecker SH, Selby DE, Palmer BM, et al. Mechanisms underlying skeletal muscle weakness in human heart failure: alterations in single fiber myosin protein content and function. Circ Heart Fail. 2009; 2:700–6. [PubMed: 19919996]
18. Poeggeler B, Pappolla MA, Hardeland R, Rassoulpour A, Hodgkins PS, Guidetti P, et al. Indole-3-propionate: a potent hydroxyl radical scavenger in rat brain. Brain Res. 1999; 815:382–8. [PubMed: 9878843]
19. Liu XH, Xin H, Hou AJ, Zhu YZ. Protective effects of leonurine in neonatal rat hypoxic cardiomyocytes and rat infarcted heart. Clin Exp Pharmacol Physiol. 2009; 36:696–703. [PubMed: 19594556]
20. Sandek A, Anker SD, von Haehling S. The gut and intestinal bacteria in chronic heart failure. Curr Drug Metab. 2009; 10:22–8. [PubMed: 19149510]
21. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006; 444:1027–31. [PubMed: 17183312]
22. Turer AT, Stevens RD, Bain JR, Muehlbauer MJ, van der Westhuizen J, Mathew JP, et al. Metabolomic profiling reveals distinct patterns of myocardial substrate use in humans with coronary artery disease or left ventricular dysfunction during surgical ischemia/reperfusion. Circulation. 2009; 119:1736–46. [PubMed: 19307475]
23. Mayr M, Liem D, Zhang J, Li X, Avliyakulov NK, Yang JI, et al. Proteomic and metabolomic analysis of cardioprotection: Interplay between protein kinase C epsilon and delta in regulating glucose metabolism of murine hearts. J Mol Cell Cardiol. 2009; 46:268–77. [PubMed: 19027023]
24. Mayr M, Yusuf S, Weir G, Chung YL, Mayr U, Yin X, et al. Combined metabolomic and proteomic analysis of human atrial fibrillation. J Am Coll Cardiol. 2008; 51:585–94. [PubMed: 18237690]

TABLE 1

Baseline Characteristics of the Study Population

Characteristics	Controls (normal)	Primary Dilated Cardiomyopathy	p
N	31	39	NA
Male /Female (%)	14 / 17 (45 / 55)	18 / 21 (46 / 54)	0.563
Age (Mean ± SD)	46.7 ± 11.1	48.1 ± 11.2	0.613
Height (cm)	171.8 ± 8.7	173.5 ± 11.2	0.491
Weight (Kg)	82.3 ± 18.8	83.4 ± 21.3	0.826
BMI (Kg/m^2)	27.7 ± 5.3	27.5 ± 5.6	0.869
Heart rate (bpm)	73.1 ± 12.1	79.6 ± 15.8	0.063
Systolic BP (mmHg)	118.8 ± 9.5	110.4 ± 14.3	0.007
Diastolic BP (mmHg)	74.1 ± 7.9	69.6 ± 12.6	0.088
Normal Sinus Rhythm (%)	31 (100)	12 (31)	<0.001
Current cigarette smoking (%)	3 (10)	0 (0)	0.077
Class I – II (%)	0 (0) (asymptmatic)	18 (46)	<0.001
Class III-IV (%)	0 (0)	21 (54)	
B Blockers (%)	0 (0)	32 (84)	<0.001
ACE – Inhibitors (%)	1 (3)	26 (68)	<0.001
ATR blockers (%)	0 (0)	3 (8)	0.176
Aldosterone receptor blockers (%)	0 (0)	13 (34)	<0.001
Furosemide (%)	0 (0)	26 (68)	<0.001
Digoxin (%)	0 (0)	16 (42)	<0.001
ST (mm)	9.2 ± 1.6	9.6 ± 1.7	0.440
PWT (mm)	9.2 ± 1.6	10.2 ± 1.7	0.023
LVEDD (mm)	45.3 ± 4.5	62.3 ± 11.9	0.0001*
LVESD (mm)	32.2 ± 5.3	51.1 ± 10.4	0.0003
LVEF (%)	63.9 ± 3.4	22.9 ± 6.8	0.0001*
LV Mass (g)	178.1 ± 49.2	332.9 ± 143.5	0.0001*
LV Mass index (g/m^2)	87.7 ± 18.5	165.6 ± 58.7	0.0001*

Abbreviations: SD: Standard deviation; cm: Centimeter; Kg: Kilogram; BMI (Kg/m^2): Body mass index in Kilogram per meter square; bpm: Beats per minute; BP: Blood pressure; ACE: Angiotensin-1 converting enzyme-1; ATR: Angiotensin II receptor; ST: Septal thickness; PWT: Posterior wall thickness; LVEDD: Left ventricular end diastolic diameter; LVESD: Left ventricular end systolic diameter; LVEF: Left ventricular ejection fraction; LV Mass; left ventricular mass

* Unequal variance, p values by Welch's approximation.

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TABLE 2

Plasma metabolites increased in patients with primary dilated cardiomyopathy

HMDB ID	Super Pathway	Pathway	Name	DCM / Control	p	q
HMDB03681	Amino acid	Guanidino and acetamido metabolism	4-acetamidobutanoate	2.2	6.33947E-06	0.0004
HMDB04827	Amino acid	Urea cycle; arginine-, proline-, metabolism	Stachydrine	2.03	0.0033	0.0112
HMDB00479, HMDB00001	Amino acid	Histidine metabolism	3-methylhistidine	1.75	0.0048	0.0145
HMDB03339	Amino acid	Glutamate metabolism	Glutamate	1.73	0.002	0.0087
	Amino acid	Glutamate metabolism	Pyroglutamine	1.66	0.024	0.0431
HMDB06344	Amino acid	Phenylalanine & tyrosine metabolism	Phenylacetylglutamine	1.64	0.0116	0.0256
	Amino acid	Tryptophan metabolism	C-glycosyltryptophan	1.63	0.0001	0.0015
HMDB00755	Amino acid	Phenylalanine & tyrosine metabolism	3-(4-hydroxyphenyl)lactate	1.63	0.0003	0.0024
HMDB00064	Amino acid	Creatine metabolism	Creatine	1.56	0.0294	0.0463
HMDB00407	Amino acid	Valine, leucine and isoleucine metabolism	Alpha-hydroxyisovalerate	1.49	0.0127	0.0269
HMDB01434	Amino acid	Phenylalanine & tyrosine metabolism	3-methoxytyrosine	1.44	0.0134	0.0275
HMDB00008	Amino acid	Cysteine, methionine, SAM, taurine metabolism	2-hydroxybutyrate (AHB)	1.38	0.0244	0.0431
HMDB00684	Amino acid	Tryptophan metabolism	Kynurenine	1.33	0.0067	0.0181
HMDB00336 HMDB00023	Amino acid	Valine, leucine and isoleucine metabolism	3-hydroxyisobutyrate	1.24	0.0276	0.0446
HMDB00158	Amino acid	Phenylalanine & tyrosine metabolism	Tyrosine	1.17	0.0288	0.046
HMDB00159	Amino acid	Phenylalanine & tyrosine metabolism	Phenylalanine	1.14	0.0211	0.0397
HMDB00613	Carbohydrate	Aminosugars metabolism	Erythronate	1.37	0.0016	0.0073
HMDB00243	Carbohydrate	Glycolysis, gluconeogenesis, pyruvate metabolism	Pyruvate	1.31	0.0261	0.0442
HMDB00169	Carbohydrate	Fructose, mannose, galactose, starch, and sucrose metabolism	Mannose	1.29	0.0063	0.0173

HMDB ID	Super Pathway	Pathway	Name	DCM / Control	p	q
HMDB00054	Cofactors and vitamins	Hemoglobin and porphyrin metabolism	Bilirubin (Z,Z)	1.48	0.0336	0.0485
HMDB00208	Energy	Krebs cycle	Alpha-ketoglutarate	2.04	0.0006	0.0038
HMDB00156	Energy	Krebs cycle	Malate	1.51	0.0101	0.0233
HMDB00552	Lipid	Carnitine metabolism	Methylglutaroylcarnitine	2.77	0.0003	0.0023
	Lipid	Bile acid metabolism	Taurocholenate sulfate	2.37	0.0012	0.0063
HMDB00063	Lipid	Sterol/Steroid	Cortisol	1.8	0.0007	0.0041
	Lipid	Bile acid metabolism	Glycocholenate sulfate	1.74	0.0037	0.0119
HMDB00211	Lipid	Inositol metabolism	Myo-inositol	1.69	0.0006	0.0035
	Lipid	Sterol/Steroid	7-alpha-hydroxy-3-oxo-4-cholestenoate (7-Hoca)	1.5	0.0002	0.0023
HMDB00097	Lipid	Glycerolipid metabolism	Choline	1.27	0.0002	0.0017
	Lipid	Carnitine metabolism	3-dehydrocarnitine	1.27	0.012	0.0257
HMDB02802	Lipid	Sterol/Steroid	Cortisone	1.2	0.0308	0.0474
HMDB00201	Lipid	Carnitine metabolism	Acetylcarnitine	1.17	0.025	0.0431
HMDB00767	Nucleotide	Pyrimidine metabolism, uracil containing	Pseudouridine	1.55	3.7338E-05	0.001
HMDB00292	Nucleotide	Purine metabolism, (hypo)xanthine/inosine containing	Xanthine	1.38	0.009	0.0213
HMDB00289	Nucleotide	Purine metabolism, urate metabolism	Urate	1.15	0.027	0.0446
HMDB06695	Peptide	Dipeptide	Pro-hydroxy-pro	1.99	0.0002	0.0023
	Peptide	γ-glutamyl	Gamma-glutamylisoleucine	1.27	0.0056	0.0157
	Peptide	γ-glutamyl	Gamma-glutamyltyrosine	1.26	0.0115	0.0256
HMDB00594	Peptide	γ-glutamyl	Gamma-glutamylphenylalanine	1.24	0.0197	0.0376
	Peptide	Γ-glutamyl	Gamma-glutamylvaline	1.21	0.0276	0.0446
HMDB02994	Xenobiotics	Sugar, sugar substitute, starch	Erythritol	1.66	0.0026	0.01

Table legend: The ratio is the mean value of each metabolite in DCM group divided to that in the control group. The mean values are based on a table of median scaled data for each compound. Accordingly, each data point is divided by the median of all detected values for that compound on that day. The procedure removes any instrument drift from day-to-day. Each data point is represented as a fraction of the median value for that day. All run days thus have the same median (1.00). The mean value is the average of all the values for the data points in a group.

TABLE 3

Plasma metabolites decreased in patients with primary dilated cardiomyopathy

HMDB ID	Super Pathway	Pathway	Name	DCM / Control	p	q
HMDB00641	Amino acid	Glutamate metabolism	Glutamine	0.9	0.0082	0.0202
HMDB02302	Amino acid	Tryptophan metabolism	Indolepropionate	0.6	0.0004	0.0027
HMDB00167	Amino acid	Glycine, serine and threonine metabolism	Threonine	0.75	0.0001	0.0015
HMDB00177	Amino acid	Histidine metabolism	Histidine	0.91	0.0179	0.0352
	Lipid	Sterol/Steroid	4-androsten-3β,17β-diol monosulfate 1	0.59	0.003	0.0106
HMDB01032	Lipid	Sterol/Steroid	Dehydroisoandrosterone sulfate (DHEA-S)	0.53	0.0001	0.0011
HMDB00626	Lipid	Bile acid metabolism	Deoxycholate	0.47	0.0001	0.0015
	Lipid	Sterol/Steroid	5α-pregnan-3 β, 20 α-diol disulfate	0.34	0.0101	0.0233
	Lipid	Lysolipid	2-Linoleoylglycerophosphoethanolamine	0.61	0.0026	0.01
	Lipid	Sterol/Steroid	Epiandrosterone sulfate	0.65	0.0021	0.0088
	Lipid	Sterol/Steroid	Pregn steroid monosulfate	0.66	0.003	0.0106
	Lipid	Lysolipid	1-oleoylglycerophosphoethanolamine	0.66	0.0131	0.0273
	Lipid	Lysolipid	1-linoleoylglycerophosphocholine	0.74	0.0001	0.0011
	Lipid	Lysolipid	1-Linoleoylglycerophosphoethanolamine	0.74	0.0018	0.008
HMDB02759	Lipid	Sterol/Steroid	Androsterone sulfate	0.75	0.0055	0.0157
HMDB00772	Lipid	Long chain fatty acid	Nonadecanoate (19:0)	0.81	0.025	0.0431
HMDB00827	Lipid	Long chain fatty acid	Stearate (18:0)	0.82	0.0269	0.0446
	Lipid	Lysolipid	1-stearoylglycerophosphocholine	0.85	0.0335	0.0485
HMDB11753	Xenobiotics	Chemical	Iminodiacetate (IDA)	0.45	0.004	0.0126
HMDB01860	Xenobiotics	Xanthine metabolism	Paraxanthine	0.5	0.0021	0.0088

TABLE 4

Absence of sex –dependent differences in the plasma metabolites levels in patients with primary dilated cardiomyopathy

	Female / Male			
	Control		DCM	
Name	**Ratio**	**q**	**Ratio**	**q**
Dehydroisoandrosterone sulfate (DHEA-S)	0.46	0.0023	0.79	0.8594
4-androsten-3β,17β-diol monosulfate 1	0.34	0.0004	0.78	0.8594
Epiandrosterone sulfate	0.48	0.0151	0.77	0.826
Pregn steroid monosulfate	0.44	0.0063	0.76	0.826
Pyroglutamine	0.44	0.0075	0.66	0.4433
Urate	0.78	0.0275	0.84	0.6845

Table legend: Blue color indicates plasma metabolites that were significantly reduced in females as compared to males

TABLE 5

Sex-dependent differences in the plasma metabolites according to the phenotype

	DCM / Control			
	Females		Males	
Name	**Ratio**	**q**	**Ratio**	**q**
Dehydroisoandrosterone sulfate (DHEA-S)	0.73	0.0812	0.42	0.0117
4-androsten-3β,17β-diol monosulfate 1	0.99	0.4055	0.43	0.0114
Epiandrosterone sulfate	0.85	0.2133	0.53	0.0267
Pregn steroid monosulfate	0.91	0.2385	0.52	0.0267
1-linoleoylglycerophosphocholine	0.78	0.0774	0.69	0.0267
Acetylcarnitine	1.06	0.5834	1.33	0.0367
γ-glutamyltyrosine	1.05	0.5306	1.54	0.0300
Choline	1.25	0.0774	1.29	0.0481
γ-glutamylisoleucine	1.08	0.5127	1.51	0.0267
C-glycosyltryptophan	1.3	0.2065	2.11	0.0267
Pseudouridine	1.44	0.0774	1.69	0.0267
Myo-inositol	1.62	0.1729	1.78	0.0453
Pro-hydroxy-pro	1.84	0.1545	2.18	0.0296
4-acetamidobutanoate	1.81	0.0753	2.68	0.0367
Methylglutaroylcarnitine	2.38	0.1515	3.28	0.0367

Table legend: Blue and red colors indicate plasma metabolites that were significantly reduced or increased, respectively, in male patients with primary DCM as compared to male individuals in the control group.

TABLE 6

Plasma metabolites levels in patients with primary DCM that were not treated with a specific heart failure medication

No β blockers (N=7)	No ACE inhibitor (N=13)	No Furosemide (N=13)	No aldosterone receptor blockers (N=36)	No Digoxin (N=23)
Androsterone sulfate	1-linoleoylglycerophosphocholine	Glutamine	5alpha-pregnan-3beta,20alpha-diol disulfate	5alpha-pregnan-3beta,20alpha-diol disulfate
Dehydroisoandrosterone sulfate (DHEA-S)			Iminodiacetate (IDA)	Stearidonate (18:4n3)
Epiandrosterone sulfate			Deoxycholate	Deoxycholate
4-androsten-3beta,17beta-diol monosulfate 1			Stearidonate (18:4n3)	Iminodiacetate (IDA)
Deoxycholate			Paraxanthine	Paraxanthine
4-androsten-3beta,17beta-diol disulfate 1			2-linoleoylglycerophosphoethanolamine	Linolenate [α or γ; (18:3n3 or 6)]
Pregn steroid monosulfate			Dehydroisoandrosterone sulfate (DHEA-S)	docosapentaenoate (n3 DPA; 22:5n3)
Histidine			Indolepropionate	Dehydroisoandrosterone sulfate (DHEA-S)
Gamma-glutamylisoleucine			1-heptadecanoylglycerophosphocholine	dihomo-linoleate (20:2n6)
Acetylcarnitine			1-oleoylglycerophosphoethanolamine	epiandrosterone sulfate
Choline			Linolenate [α or γ; (18:3n3 or 6)]	2-linoleoylglycerophosphoethanolamine*
2-methylbutyroylcarnitine			γ-tocopherol	oleate (18:1n9)
Erythronate			Pregn steroid monosulfate	Linoleate (18:2n6)
Erythritol			1-linoleoylglycerophosphocholine	Margarate (17:0)
Pseudouridine			1-linoleoylglycerophosphoethanolamine	10-heptadecenoate (17:1n7)
Bilirubin (Z,Z)			Stearate (18:0)	Indolepropionate
Glycocholenate sulfate*			1-stearoylglycerophosphocholine	Pregn steroid monosulfate
Pro-hydroxy-pro			Linoleate (18:2n6)	Stearate (18:0)
4-acetamidobutanoate			Nonadecanoate (19:0)	Myristate (14:0)
Myo-inositol			Threonine	1-linoleoylglycerophosphocholine
3-methylhistidine			1-oleoylglycerophosphocholine	1-eicosatrienoylglycerophosphocholine
Taurocholenate sulfate			Glutamine	Palmitate (16:0)
Stachydrine			Phenylalanine	1-palmitoylglycerol (1-monopalmitin)

No β blockers (N=7)	No ACE inhibitor (N=13)	No Furosemide (N=13)	No aldosterone receptor blockers (N=36)	No Digoxin (N=23)
Methylglutaroylcarnitine			Choline	Nonadecanoate (19:0)
			7-alpha-hydroxy-3-oxo-4-cholestenoate (7-Hoca)	Threonine
			Erythronate	Dihomo-linolenate (20:3n3 or n6)
			Kynurenine	Glutamine
			Pseudouridine	Choline
			2-hydroxybutyrate (AHB)	7-α-hydroxy-3-oxo-4-cholestenoate (7-Hoca)
			3-(4-hydroxyphenyl)lactate	Xanthine
			C-glycosyltryptophan	Erythronate
			4-acetamidobutanoate	Pyruvate
			Pro-hydroxy-pro	Erythritol
			Creatine	Pseudouridine
			3-methylhistidine	Urea
			Myo-inositol	3-(4-hydroxyphenyl)lactate
			α-ketoglutarate	C-glycosyltryptophan
			Cortisol	Creatine
			Glutamate	Pro-hydroxy-pro
			[H]HWESASLLR[OH]	4-acetamidobutanoate
			Stachydrine	Myo-inositol
			Taurocholenate sulfate	α-ketoglutarate
			Methylglutaroylcarnitine	Cortisol
				Taurocholenate sulfate
				Methylglutaroylcarnitine

Figure legend: Blue and red colors indicate plasma metabolites that were reduced or increased, respectively, in patients with primary DCM who were not treated with a specific heart failure medication as compared to controls ($q<0.05$).