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ORIGINAL ARTICLE

Identification of Metabolite Shifts and Early Serum Predictors for Indicators of Remodelling in Diabetes and Nondiabetic Models of Cardiac Hypertrophy

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Background: Cardiac hypertrophy (CH) is the asymptomatic enlargement of ventricular walls witnessed in diabetes and hypertension, for which early metabolite differences and prediction are less stated previously. **Aim:** The aim of the study was (i) to understand the metabolic and ventricular events in diabetes and nondiabetes induced CH at the end of 2 weeks and (ii) to identify significant metabolite predictors and pathways that influence the seven metabolic and physiological responders of CH, namely, 3-hydroxybutyrate (3-HB); lactic acid; urea; and electrocardiography (ECG) waves (QRS complex, R amplitude, R-R interval, and heart rate). **Methods:** Diabetic rat models of CH using streptozotocin (40 mg/kg, i. p., single dose), and nondiabetic models using adrenaline (0.3 mg/kg, i. p, 2 weeks) were developed. Blood glucose levels, ECG, heart weight/body weight ratio, histopathological analysis, and serum metabolite analysis using gas chromatography mass spectrometry were performed at the end of 2 weeks. Strong metabolite predictors and pathways were identified using Pearson's correlation, multiple regression (MRA) and metabolite set enrichment (MSEA) analyses. **Results:** The prevalence of CH was observed through preliminary screenings at the end of 2 weeks. Galactose, leucine, erythrose, sorbitol, and valine were identified as significant ($P < 0.05$) predictors in SZ model, whereas isoleucine, galactose, leucine, inositol, and palmitic acid were identified in ADR model. However, galactose metabolism, branched-chain amino acid, and lactose degradation pathways were mapped as the highly influential apparent pathways during early CH remodeling in both the models. **Conclusion:** This study identified putative initial metabolite shifts, significant predictors pathways that can aid in forecasting, intervention, and prevention of CH.

Key words: Cardiac hypertrophy, diabetes, adrenaline, gas chromatography mass spectrometry, early predictors

INTRODUCTION

Cardiac hypertrophy (CH) is characterized by enlarged ventricles due to various physiological and pathological stressors such as chronic vigorous exercises, comorbidities like diabetes, hypertension, chronic kidney disease, obesity, and intake of anthracyclines by cancer patients.[1-3] Chronic energy deficiency due to the imbalance between glucose and fatty acid oxidation ultimately causes cardiac dysfunctions that are diagnosed only after intense cardiac impairment by echocardiography or sudden cardiac death (SCD), for which adrenaline is administered during emergency with typical treatments that include β-adrenergic receptor blockers and angiotensin-II receptor antagonists.[4,5] Electrocardiography (ECG) parameters were reported as the inexpensive markers of myocardial global electric heterogeneity that can predict the ventricular structural and functional abnormalities and SCD. As lead II is the elemental ECG wave for CH,[6,7] this study focused on the QRS complex, R amplitude, R-R interval, heart rate, and screening of β-hydroxybutyrate (3-HB), lactic acid (LA), urea as the seven responders of metabolic and physiological homeostasis.[8-10] Hence, the objective of this study was to screen the metabolite shifts and identify early predictors for the seven responders of CH.

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MATERIALS AND METHODS

Chemicals

All chemicals and reagents used were of analytical grade from Hi Media Pvt Ltd., India. Streptozotocin (SZ) was purchased from SRL Pvt. Ltd., India. *N*-Trimethylsilyl-*N*-methyl trifluoroacetamide (MSTFA) and methoxyamine hydrochloride were purchased from Sigma-Aldrich. Adrenaline (Adrenicure 1 mg/ml injection ampoules) was purchased ethically from the licensed pharmacy shop at Coimbatore.

Experimental rats

Male Sprague Dawley rats weighing between 200 and 210 g procured after ethical clearance (CPCSEA/No: 399/2018/IAEC) were acclimatized for 3 days under controlled temperature of 29°C ± 5°C, humidity at 55% ± 5%, and 12 h of light/dark cycles. They were divided into two groups with five rats per group and were subjected to induction and development of diabetes for 2 weeks as follows and after which the fasting blood glucose was measured using AccuCheck Active glucometer.

- Group 1 – normal (control – NOR)
- Group 2 – SZ (SZ – 40 mg/kg, i. p., single dose, 2 weeks)[11]
- Group 3 – adrenaline (ADR – 0.3 mg/kg, i. p, 2 weeks).[4]

Electrocardiography analysis of cardiac hypertrophy underlying DC

To monitor the cardiac function *in vivo* after 2 weeks, ECG analysis of the conventional bipolar limb lead II using BITalino ECG Sensor-OpenSignals (*r*) evolution software was performed for 6 min in unanesthetized rats for recording the changes of QRS complex, R amplitude, R–R interval, and pulse/heart rate (HR). The percentage changes in these parameters were calculated as: ([ECG parameter value in SZ-in NOR]/[in NOR] × 100).[5,12]

Assessment of heart weight/body weight ratio

The enlarged heart sizes in each rat models were determined using the heart weight (HW)/body weight (BW) ratio.[5]

Histopathological analysis of left and right ventricles

The heart tissues were excised and initially preserved in 10% formalin until their left and right ventricles were processed and stained with hematoxylin and eosin (H & E) to examine their cellular architecture at 40X.[13]

Gas chromatography-mass spectroscopy analysis of serum metabolites

Serum was isolated from the blood through cardiac-puncture after overnight fasting and derivatized as per the modified protocol.[14] Briefly, 100 µl serum was precipitated using 250 µl acetonitrile, evaporated to dryness using N_2 gas. 20 mg/ml methoxylamine hydrochloride dissolved in pyridine was added and incubated at 70°C for 60 min followed by 50 µl MSTFA and incubated 40°C for 90 min. 1 µl derivatized sample was injected into the inlet port at 10:1 split mode and analyzed using a Shimadzu GC-2010 plus gas chromatography instrument coupled to a Shimadzu QP2010 mass spectrometer (Shimadzu, Japan). Helium served as carrier gas at flow rate of 1 ml/min with the initial temperature as 100°C for 4 min that was elevated to 270°C at the rate of 5°C/min. The temperatures of injection were 280°C, interface was 250°C, and ion source was 200°C with a solvent delay of 9 min. The same procedure was followed for the reference compound ribitol. MS was operated in electron ionisation mode of 70 eV, and scan range was between 35 and 800 m/z followed by the identification of metabolites based on NIST and WILEY mass library.

Data analysis

All the data were expressed as mean ± standard error of the mean with significance at $P < 0.05$ using analysis of variance.[5,14] Pearson's correlation analysis along with pattern search analysis was performed to identify metabolites strongly related between themselves and with the seven responders (QRS complex, R-amplitude, R-R interval, HR, 3-HB, LA, and urea) of this study. The list of a few strongly related metabolites was then subjected to multiple regression analysis (MRA) to identify the significantly potent predictor metabolite for each of the seven responders, respectively. Similarly, metabolite set enrichment analysis (MSEA using SMPDB library) was also performed to identify highly significant probable metabolic pathways, in which the identified predictors participate and might exert cumulative effects during early remodeling. MRA was performed using IBM SPSS 26.0, and the Pearson's correlation, pattern hunting, and MSEA were performed using MetaboAnalyst 4.0.[15,16]

RESULTS

Electrocardiography screening for cardiac hypertrophy in DC

Primary CH features such as widened QRS complex, elevated R-amplitude, and prolonged R-R interval were observed in SZ and ADR administered rats when compared to normal as seen in Figure 1 and Table 1. Although the changes in QRS complex were insignificant statistically, other ECG parameters of this study were significant, thereby indicating the commencement and early stage of CH at the end of 2 weeks.

Table 1: Screening of cardiac hypertrophy characteristics and physiological responders using electrocardiography

Groups	QRS complex (ms)	Percentage change	R amplitude (mV)	Percentage change	R-R interval (ms)	Percentage change	HR (bpm)	Percentage change
I - NOR	19.30±0.52	-	0.43±0.05	-	162.49±2.93	-	369.00±1.77	-
II - SZ	23.00±3.25[a]	19.45↑	0.62±0.05[a],*	45.32↑	236.00±4.71[a],*	45.24↑	254.00±5.12[a],*	31.14↓
III - ADR	22.80±1.65[b]	18.39↑	0.68±0.05[b],*	57.94↑	201.00±4.19[b],*	23.70↑	299.00±6.10[b],*	19.15↓

*Statistical significance (P<0.05), Group comparison=[a]NOR versus SZ; [b]NOR versus ADR. Values as the mean±SE of 5 samples per group. The percentage changes in each ECG parameters are indicated by red (increase) and blue (decrease) arrows. HR=Heart rate; NOR=Normal; SZ=Streptozotocin; ADR=Adrenaline; ECG=Electrocardiography; SE=Standard error; QRS=Ventricular depolarization

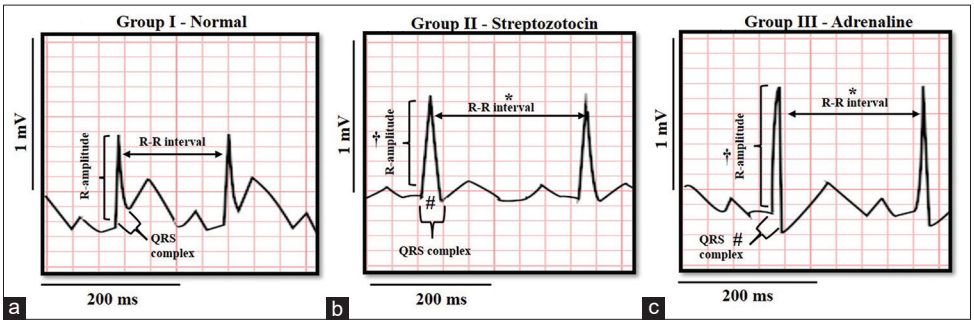

Figure 1: (a-c) Graphical representation (scaled to the real-time raw electrocardiography tracings-Lead II) of cardiac hypertrophic events. Streptozotocin administered rats show widened QRS complex, elevated R-amplitude, prolonged R-R interval (reduced pulse/heart rate) indicating ventricular dysfunction due to impaired impulse conductivity

Blood glucose estimation and hypertrophic index (heart weight/body weight ratio)

Blood glucose was significantly increased at the end of 2 weeks accompanied by elevated HW in streptozotocin and adrenaline administered rats. Though statistically insignificant, considerable reduction in BW was observed in both groups accompanied by significant mild increase in HW/BW ratio [Table 2] and enlarged heart sizes [figure 2].

Histopathological analysis of ventricles

H and E staining revealed the distorted and degenerating cellular architecture of the left and right ventricles in diabetic SZ (Group II) and nondiabetic ADR (Group III) models when compared to the normal heart (Group I) as seen in Figure 3. Although both models express thick myocardial fibers and hypertrophic nuclei with abnormal connective tissue separations, the different pathophysiological impacts of SZ and ADR can be well visualized with the uniquely distorted cellular architecture by each inducer.

Metabolite profiling and correlation analysis to identify relationship between metabolites and the responders

Upon comparing the serum of normal (NOR) with the sera of SZ and adrenaline (ADR) administered rats, gas chromatography mass spectrometry (GC-MS) analysis revealed a panel of serum metabolites, wherein L-valine, L-isoleucine, L-alanine, L-proline, and D-mannose were comparatively low in SZ-induced diabetic rats, whereas L-proline was the only prominently low serum metabolite in ADR model. Elevated serum glucose levels along with the apparent presence of D-ribose, D-erythrose, and D-sorbitol were also observed in SZ-administered rats, whereas L-aspartic acid, L-glutamic acid, D-ribose, D-succinic acid, D-sorbitol, butanoic acid, and myristamide were seen observed highly elevated in ADR model when compared to normal serum as shown in Figure 4. Figure 5 shows the Pearson's correlation for the relationship strength among metabolites and between the seven responders of this study. Their better visualization through pattern hunting as indicated in green boxes shown in Supplementary Figures 1 and 2 indicate metabolites that were strongly correlated with each metabolic responder. Each responder was considered as independent variable and proceeded with multiple regression analysis to identify a single potent putative predictor for each of the seven dependent variable responders.

Identification of specific putative predictors for the metabolic responders using multiple regression analysis

Among the metabolites listed in Figure 4, galactose, leucine, erythrose, sorbitol, and valine were found to be the highly potent predictors for the SZ-induced diabetes-associated CH, whereas isoleucine, galactose, leucine, inositol, and palmitic

acid (PA) were identified as significant predictor metabolites for ADR-induced CH at the end of 2 weeks. In SZ model, all predictors except galactose exhibited very good regression fit ($R^2 \geq 0.80$), but due to the strong significance ($P < 0.05$) by all these five predictors, they were further considered for MSEA similar to the ADR model in order to check pathways that are probably influenced by these predictors.

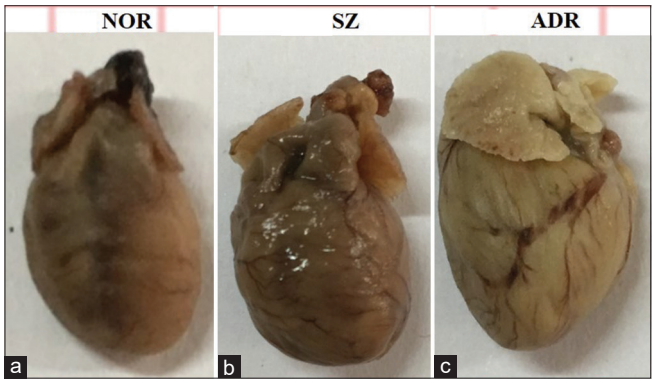

Figure 2: (a-c) Heart sizes of experimental rats. At the end of 2 weeks, when compared to normal, the Streptozotocin and ADR administered rats represented enlarged and inflamed heart tissue, especially in the ventricular regions (scaled in graph)

MSEA of predictors for identification of enriched pathways

Galactose metabolisms, branched-chain amino acid (BCAA), and lactose degradation pathways were mapped as the highly impacted and enriched pathways by the five significant predictors of each CH models that, besides, their varying enrichment scores indicate the metabolic pathways affected by the two inducers vary that might have effective roles and that can be affected during SZ-induced diabetes-associated CH [Figure 5].

DISCUSSION

CH is the initial remodelling stage in diabetes-induced cardiomyopathy (diabetic cardiomyopathy) and a crucial clinical subset of cardiac diseases including myocardial ischemia or infarction developed due to various clinical and physiological conditions. CH initially develops as a reversible cellular adaptative condition to combat physiologically or pathologically induced stress which transforms into irreversible cardiac abnormality, wherein SCD has been reported as one of the common end points. CH has been previously studied extensively in terms of oxidative and reductive stress and

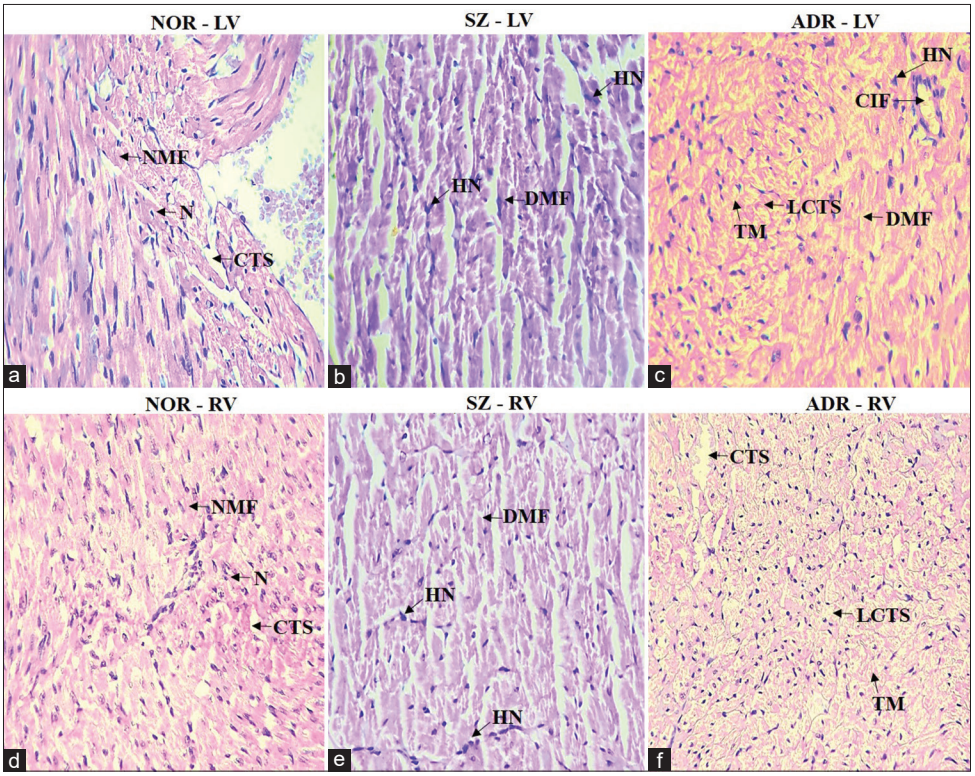

Figure 3: (a-f) Histopathological analysis of ventricles. Streptozotocin administered CH rats show distorted muscle fibers and hypertrophied nuclei in left and right ventricles. ADR administered rats reveal impaired cardiac architecture different from Streptozotocin group when compared to normal. In the figure, nuclei (N), muscle fibers and connective tissue separations

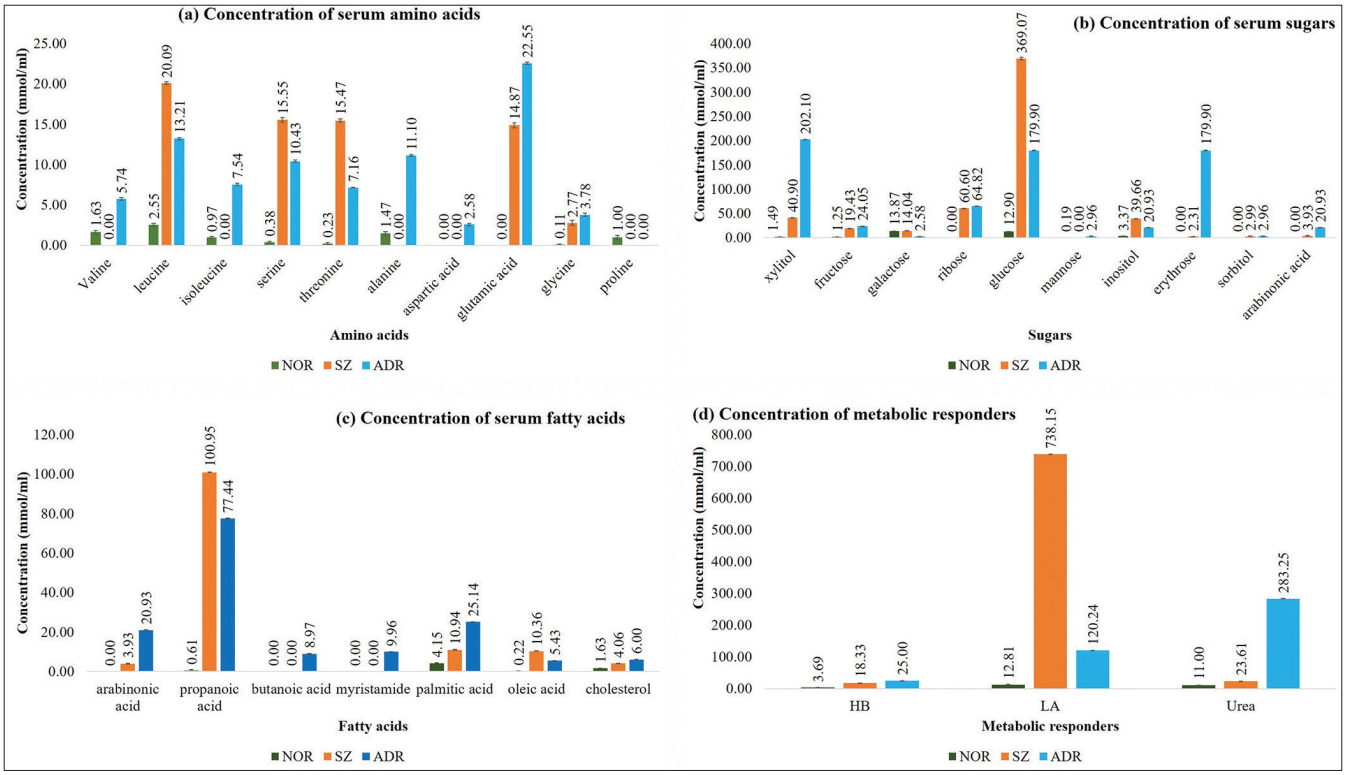

Figure 4: (a-d) Concentrations of serum metabolites identified by gas chromatography mass spectrometry. The figure shows the concentrations of amino acids, sugar and lipid metabolites along with the metabolic responders 3-HB, lactic acid and urea among the experimental rats

Table 2: Blood glucose levels and hypertrophic indices among experimental rats

Groups	Blood glucose (mg/dL)	BW (g)	HW (mg)	HW/BW ratio
I - NOR	81.00±3.74	203.00±4.47	0.892±0.010	4.39±0.0001
II - SZ	382.20±8.78[a],*	200.20±3.13[a]	0.930±0.005[a],*	4.64±8.08E-05[a]
III - ADR	178.92±4.22[b],*	192.64±6.35[b]	1.132±0.080[b],*	5.87±0.38[b],*

*Statistical significance (P<0.05), Group comparison=[a]NOR versus SZ; [b]NOR versus ADR. The mean±SE of 5 samples per group. BW=Body weight; HW=Heart weight; NOR=Normal; SZ=Streptozotocin; ADR=Adrenaline; SE=Standard error

their regulation by Nrf-2-associated antioxidant systems.[8,17,18] Recently, deep learning computational methods and modern statistical methods combined with Artificial Intelligence were used in precision medicine to accurately predict blood sugar levels from ECG observations itself.[19] Thus, the influences of metabolite perturbations that underlie disease development and progression to cardiovascular dysfunction, if predicted using metabolites, can portend the commencement of CH much earlier and those metabolites can be called as early predictors.

This study used two modes for CH development; one was SZ- provoked diabetes-induced cardiac impairments and the other was adrenaline (ADR) administration which was considered as nondiabetes model that contributes to CH. SZ has been previously shown to induce changes in the cardiac electrophysiology and ventricular events after its 5th day of administration,[20] yet, its earlier metabolic events before and after day 5 before the advanced stage are scarcely understood. Adrenaline (ADR) or epinephrine is a nonselective α-and β-adrenergic agonist administered as a life support along with cardiopulmonary resuscitation during cardiac emergencies. ADR has been previously associated with increased myocardial dysfunction and cerebral ischemia in response to postresuscitation and even in vigorous exercises that in turn were chronically linked with SCD.[18]

In this study, impaired QRS complex, R-amplitude, R-R interval, heart rate (HR), abnormal levels of 3-hydroxybutyrate (3-HB), LA, and urea were considered as applicable responders for metabolic stress during CH. We hypothesized that an easily traceable serum metabolite with strong influence for those seven responders at the end of 2 weeks can possibly become early predictors that influence and showcase the progression of cardiac remodeling. The unique presence of elevated ribose, erythrose, and sorbitol along with traces of valine, isoleucine, alanine, proline, and mannose in SZ model, whereas increased aspartic acid, glutamic acid, ribose, succinic acid, sorbitol, butanoic acid,

and myristamide accompanied by a lower concentration of proline in ADR model witnessed in this study show the metabolite profile variations between the two modes of CH routes during early events. However, as seen in Tables 3 and 4, galactose, leucine, erythrose, sorbitol, and valine of SZ model, and isoleucine, galactose, leucine, inositol, and PA of ADR model were identified as significant predictor metabolites for their respective responders during early CH events that were confirmed primarily through ECG and hypertrophic indices.

Although the blood glucose was found elevated in both SZ and ADR models, the prominent hyperglycemic condition was observed in SZ primarily due to its direct toxic potential on pancreas that affects insulin secretion[13] unlike ADR that increases blood glucose through secondary responses such as enhanced glycogenolysis and gluconeogenesis.[8] ADR though well established to develop hypertension-based CH models[4] was not evaluated for high blood-pressure analysis in this study because the objective was only to understand the metabolite shifts and predictors after ADR administration irrespective of blood pressure effects with comparison to SZ-induced diabetes-based CH model at the end of 2 weeks. In addition, this study showcased the three most impacted metabolic pathways, thereby hinting the convergence during metabolic remodeling underlying two different pathophysiology by diabetes (SZ model) and nondiabetes (ADR model) of CH progression and intervention.

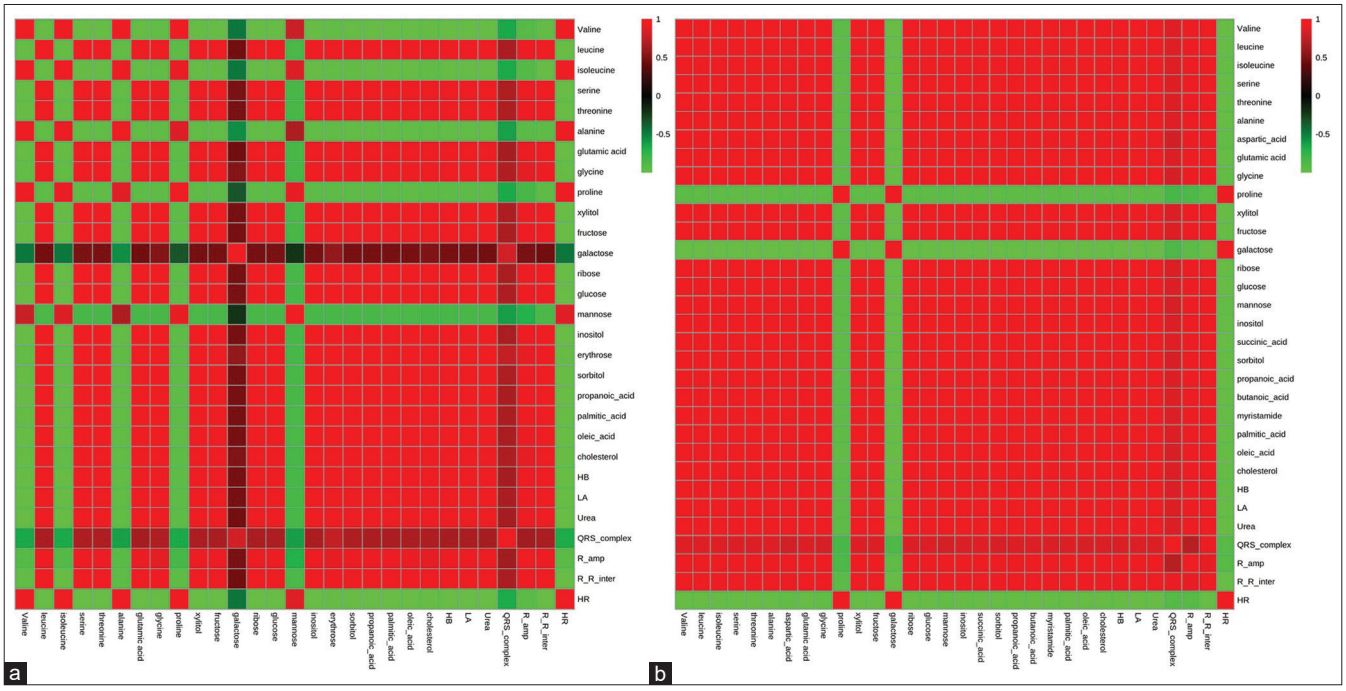

Figure 5: Correlation heat map of metabolites and metabolic responders in Streptozotocin and ADR models. Pearson's Correlation analysis revealed strong correlation of the unique finger-print metabolites in Streptozotocin (a) and ADR (b) administered rats. Green color indicates negative correlation, whereas red indicates positive correlation ranging between −1 and +1, respectively

Table 3: Predictors for metabolic responders in streptozotocin induced cardiac hypertrophy models

Metabolic responders	Potent predictor metabolite	Prediction equation	Predictor - Linear model fitness and its significance
QRS complex	Galactose	y=−104.964+8.951x	R^2=0.51; P=0.005
R-amplitude	Leucine	y=0.187+0.077x	R^2=0.88; P=0.031
R-R interval	Erythrose	y=398.828+20.671x	R^2=0.96; P=0.0002
HR/pulse rate	Sorbitol	y=429.441+30.114x	R^2=0.95; P=0.0002
3-HB	Valine	y=19.317+3.894x	R^2=0.99; P=0.000007
LA	Valine	y=736.744+204.222x	R^2=0.99; P=0.000004
Urea	Valine	y=23.057+4.199x	R^2=0.93; P=0.032

*Statistical significance (P<0.05). The metabolites identified as predictor (independent variable) for the respective metabolic responders (dependent variable) during SZ induced earlier stages of diabetes based CH at the end of 2 weeks. The table also displays the linear regression model fitness for each predictor metabolite. HR=Heart rate; HB=β-hydroxybutyrate; LA=Lactic acid; SZ=Streptozotocin; CH=Cardiac hypertrophy; QRS=Ventricular depolarization

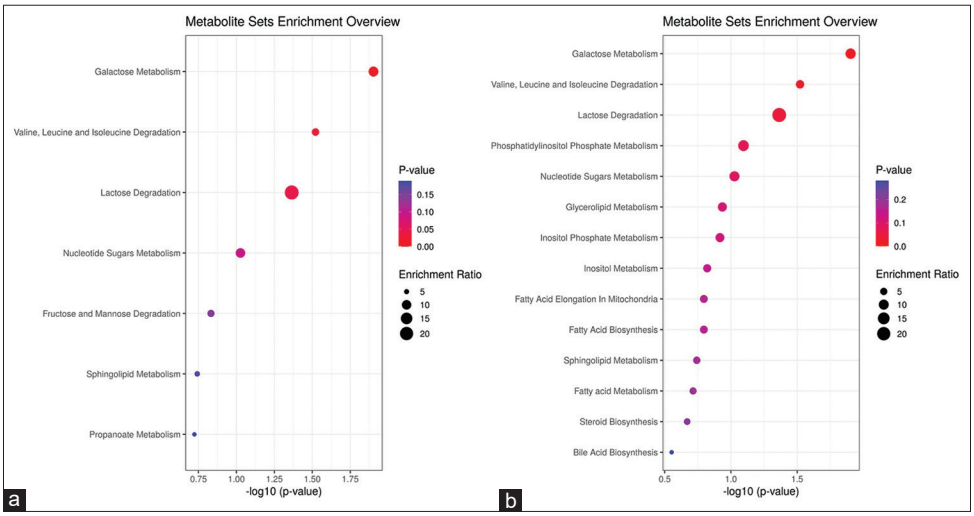

Figure 6: (a and b) Metabolite set enrichment analysis for predictors. Lactose degradation identified as the highly influential pathway followed by galactose metabolism as the most significant, accompanied by BCAA (valine, leucine, and isoleucine) degradation. Though other metabolic pathways are represented by low enrichment and significant values, they may have roles during remodeling events

Table 4: Predictors for metabolic responders in adrenaline induced cardiac hypertrophy models

Metabolic responders	Potent predictor metabolite	Prediction equation	Predictor - Linear model fitness and its significance
QRS complex	isoleucine	y=91.947+7.377x	R^2=0.97; P=0.007*
R-amplitude	galactose	y=−1.256+0.620x	R^2=0.90; P=0.018*
R-R interval	leucine	y=28.054+20.092x	R^2=0.99; P=0.0003*
HR/pulse rate	inositol	y=418.559+1.918x	R^2=0.89; P=0.001*
3-HB	palmitic acid	y=−6.446+0.735x	R^2=1.00; P=1.0004E-7*
LA	inositol	y=96.576+2.922x	R^2=1.00; P=2.2068E-7*
Urea	inositol	y=200.355+6.451x	R^2=1.00; P=4.5625E-8*

*Statistical significance (P<0.05). The metabolites identified as predictor (independent variable) for the respective metabolic responders (dependent variable) during ADR induced CH at the end of 2 weeks. The table also displays the linear regression model fitness for each predictor metabolite. HR=Heart rate; HB=β-hydroxybutyrate; LA=Lactic acid; ADR=Adrenaline; CH=Cardiac hypertrophy; QRS=Ventricular depolarization

Galactose and its metabolism

In this study, serum galactose in SZ and ADR models had been identified as a predictor metabolite that influences ventricular depolarization-QRS complex and R amplitude, respectively. QRS complex abnormalities of ventricular dysfunction are seen in Fabry's disease, an α-galactosidase deficiency that manifests as CH. Galactose was previously reported in association with aggravated inflammation, calcium signaling, ROS, oxidative stress, apoptosis, advanced glycation end products (AGEs) that lead to cardiac fibrosis, hypertrophy, senescence, and dysfunction.[21,22] This correlation between the early galactose metabolic perturbations seen in the serum and the ventricular abnormalities of CH is a significant finding of this study.

Lactose degradation pathway

Besides, the galactose-induced metabolic perturbations aforementioned, lactose degradation pathway had been mapped as the most significantly impacted pathway in SZ and ADR models of CH. Lactose overconsumption, impairment of enzymes of lactose degradation either due to inherited or intestinal damage from viral infections, lactose intolerance, lactose malabsorption, shifts in lactose degradation products, namely, galactose and glucose are all hallmark of diabetes, hypertension, and CH pathophysiology.[23] Thus, this study recommends detailed exploration of the lactose degradation pathway contributing to cardiac remodeling.

Branched chain amino acid

Among the three BCAA, leucine and valine were identified as predictors for R-amplitude and urea, respectively, in SZ model, whereas isoleucine and leucine as predictors for QRS complex and R-R interval, respectively, in ADR model. Valine and isoleucine were found elevated in ADR model, whereas trace in SZ model was accompanied by elevated levels of leucine in both the models. Degradation pathway of valine,

leucine, and isoleucine, the BCAAs have been mapped as highly impacted pathway by MSEA in this study. Leucine levels have been previously shown to reduce the occurrence of cardiomyocyte apoptosis in cancer-cachexia model and thereby it could be the reason why leucine has been identified as a significant regulator of ventricular depolarization events (R-amplitude and R-R interval) as seen in this study.

Earlier studies have reported defective isoleucine metabolism in a condition called propionic aciduria that manifests left ventricular dysfunction indicated by prolonged QRS complex. This hereby advocates the identification of isoleucine as a predictor in the present study which warrants direct investigation of it in CH pathophysiology. Even though plasma valine levels are reported to increase in the type 2 diabetes after 4 weeks of high-fat diet, elevated leucine decreased the valine and isoleucine levels in this study. It may be probably due to the excess utilization of ketone bodies as rightly obtained predictor-responder "relationship" between the valine and 3-HB. The low serum concentration of the predictor, valine in SZ-administered diabetic rats after 2 weeks may be due to the influence of gut microbial community during diabetes and hence needs further analysis of the same. A previous study highlighted the relationship between valine oxidation and blood urea nitrogen. To the best of our knowledge, this is the first report of an association between the valine with urea in rat models identified through GC-MS-based MRA and MSEA. Similarly, association between valine and LA in a previous study had been identified as putative biomarker for brain tumour and abscess discrimination.[24-26]

Erythrose

Primarily, erythrose-4-phosphate has been associated with Nrf-2-mediated regulation of metabolic enzymes and reducing equivalents such as NADPH that play a key role in hypoxia and CH. Rightly, in this study too, erythrose has been mapped as a predictor of R-R interval in SZ model.[25]

Sorbitol and Inositol

This study identified sorbitol as a predictor of heart rate (HR) in SZ model and inositol in ADR model. Chronically increased blood glucose activates the sorbitol pathway, thereby converting glucose to sorbitol by aldose reductase. High sorbitol concentration contributes to the release AGEs, namely, 3-deoxyglucosone and glyoxal production. These enhance collagen accumulation and cardiac fibrosis which in turn impairs cardiac electrical conductivity and affects heart rate (HR) possibly through PKA/PKC-β signaling mechanisms[8] whose detailed mechanistic investigation is warranted. Inositol besides as a predictor for HR was also identified as a predictor for LA and urea. Inositol 1,4,5 triphosphate (IP3) receptor signaling has been enormously studied earlier in terms of cardiac excitation-contraction coupling mechanisms and arrhythmias through α/β-adrenergic receptors that hereby explains the background of inositol as a predictor of HR or cardiac rhythm. Inositol and urea were previously related to each other by early growth response protein-1 that can be activated by urea which in turn stimulated inositol phosphates. During acute kidney injury, increased blood urea was accompanied by elevated levels of an enzyme, myoinositol oxygenase that catabolizes myoinositol. Similarly, in a previous study, the levels of inositol and lactate were found elevated during fetal metabolism, a metabolic characteristic in CH. A common event between the responder LA and predictor inositol is the insulin-glucose signaling mechanism as identified in earlier studies,[27-29] and therefore, this study recommends a direct investigation between the responders and inositol in terms of CH pathophysiology.

Palmitic acid

Elevated PA in ADR-administered rats was identified as a predictor of 3-HB. PA, a long-chain saturated fatty acid synthesized from acetyl coenzyme A and malonyl coenzyme A by fatty acid synthase had been previously shown to be affected by ADR during which increased oxidation of PA was reported to occur through ketone bodies like 3-HB. Palmitate inhibition by ketone bodies, especially 3-HB, was reported as competitors of myocardium fuels[30] that herein explains their relationship, thereby validating the prediction of 3-HB using palmitate levels in serum of ADR induced CH.

CONCLUSION

Hence, this study identified metabolites and pathways with crucial interplay in CH developed by diabetic (streptozotocin model) and non-diabetic (adrenaline model) conditions vary. The linear equation for each predictor–responder model along with the concentration of the predictor metabolite can be used to predict the status of the respective responder; thereby, we can decipher the stages of remodeling that occurs gradually and sequentially. The mapping of the three common metabolic pathways by the different predictor metabolites for their respective responders herein indicates that though the modes of CH pathophysiology for diabetic (SZ) and nondiabetic (adrenaline) vary, yet they converge mechanistically. This fundamental yet key observation makes it promising for future studies with integrated approach of metabolomics and machine-learning statistical methods. Such as approach can predict the levels of pathophysiological responders merely based on the concentration of predictor metabolites. This may enable detection and forecasting of early events of CH. Besides, the mechanistic details of how these early changes in metabolite patterns translate into CH pathophysiology can be explored more extensively.

Acknowledgments

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Conflicts of interest

There are no conflicts of interest.

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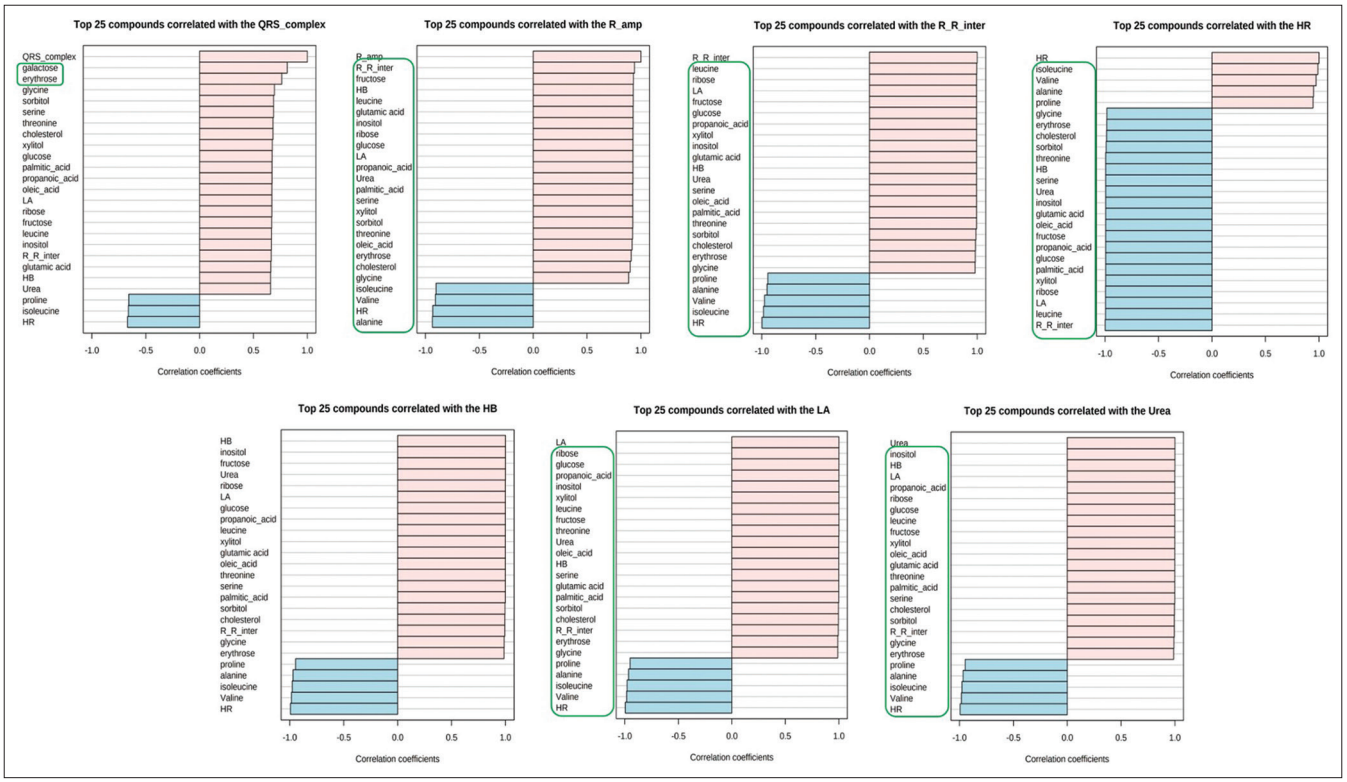
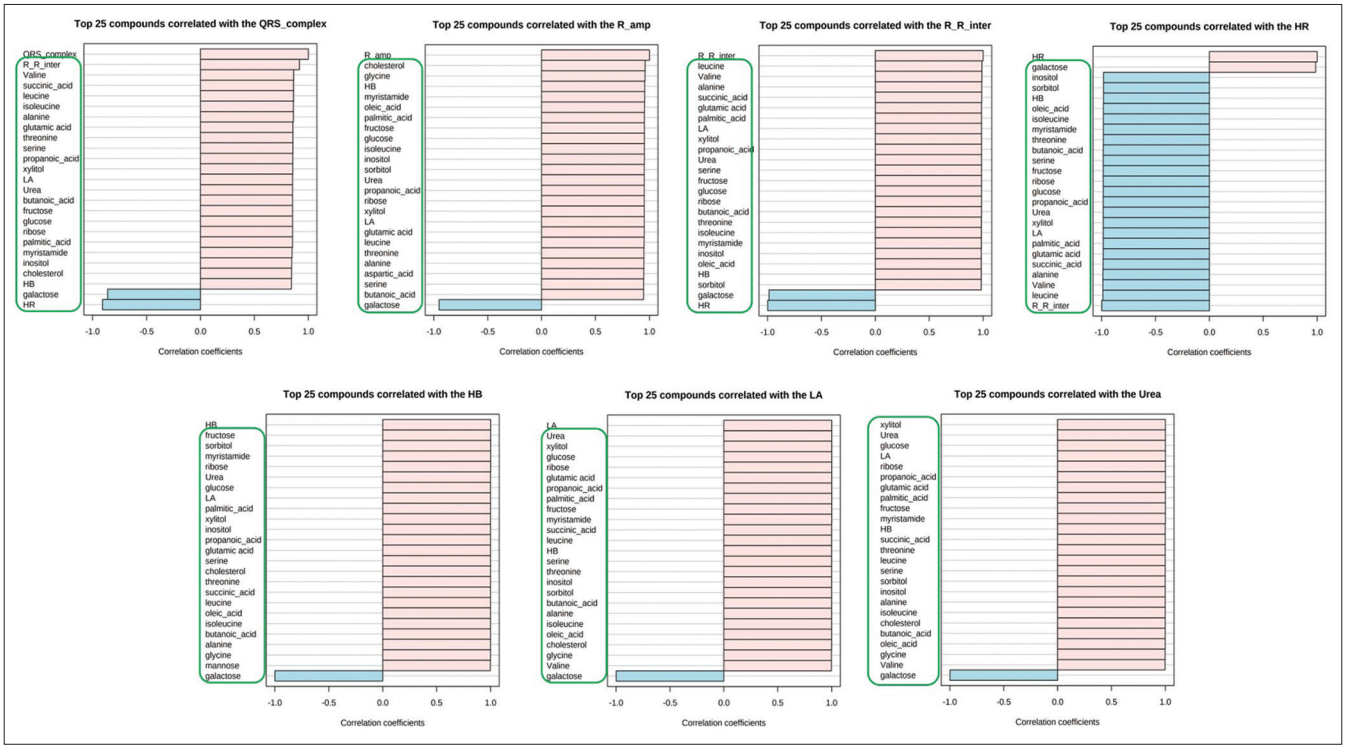


Supplementary Figure 1: Pattern hunting to identify metabolites strongly related with the physiological and metabolic responders in streptozotocin-induced cardiomyopathic rats. In the figure, blue indicates negative correlation and pink indicates positive correlation



Supplementary Figure 2: Pattern hunting to identify metabolites strongly related with the physiological and metabolic responders in ADR-induced cardiomyopathic rats. In the figure, blue indicates negative correlation and pink indicates positive correlation