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Original article

Untargeted GC–MS investigation of serum metabolomics of coronary artery disease patients

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ABSTRACT

Recent advances in metabolomics provide tools to investigate human metabolome in order to establish new parameters to study different approaches towards diagnostics, diseases and their treatment. The present study focused on the untargeted identification of metabolites in serum of patients with coronary artery disease who were under treatment at the time of sample collection. AUCs (Area Under the Curves) from different peaks were considered for the analysis and comparison purposes. The metabolome was studied using GC–MS (Gas Chromatography Mass Spectrometry) and the metabolites were identified with NIST (The National Institute of Standards and Technology) and Wiley library matches. A total of 17 metabolites were identified and focused on to compare with the metabolome of healthy individuals. T test analysis found significant differences in alanine, malonic acid, ribitol, D-glucose, mannose ($P < 0.001$), acetohydroxamic acid, N-carboxyglycine, and aminobutyrate ($P < 0.05$). Principal Component Analysis of serum metabolites data found three components out of 17 metabolites; RC1 (Acetohydroxamic acid, alanine, D-glucose, malonic acid, mannose, N-carboxy glycine and ribitol), RC2 (Heptadecanoic acid, hexadecanoic acid, octadecanoic acid and Trans-9-octadecanoic acid), RC3 (Aminobutyrate, D-sorbit, gamma lactone, valine, benzene propanoic acid and lactic acid). No correlation was found among the components.

1. Introduction

During the recent past, metabolomics has emerged as a new investigation tool in the areas including, but not limited to, health and diseases. Collective investigation of metabolites in different body fluids allows identification of novel biomarkers of diseases, metabolomics fingerprints associated with diseased conditions, drug toxicity, diagnostics, etc. (Spratlin et al., 2009; Patel and Ahmed, 2015; Rzeznik et al., 2017).

Cerebrospinal fluid, blood, saliva and urine are the common biofluids in case of human metabolomics studies. Serum metabolome has been studied in detail and several methods and tools to study the same has been proposed by the researchers globally (Psychogios et al., 2011; Lau et al., 2018). Published reports indicate the possibility to identify acute metabolomics changes in human serum (Rachakonda et al., 2014; Stander et al., 2018) that also extends to chronic metabolomics changes in certain conditions (Joseloff et al. 2014). Biomarker identification through metabolomics has gained recognition not only in diagnostics but in therapeutics (Lanznaster et al. 2018) and forensic toxicology as well (Steuer, Brockbals, and Kraemer 2019).

Coronary artery disease (CAD) is one of the most common diseases and the leading cause of death globally (Consortium et al., 2013). Several risk factors have been associated with the increase risk of CAD including high cholesterol levels, hypertension, diabetes, aging, and smoking (Jensen et al. 2014). The relationship

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between CAD and some metabolites such as cysteine, cholesterol, and triglycerides, have already been established (Jensen et al. 2014). Other studies investigated several metabolites that could be a source of potential biomarkers of CAD (Li et al., 2017; Gottdiener et al., 2000; Koeth et al., 2013; Mente et al., 2015). Nevertheless, yet there is no specific and definitive metabolite biomarker for CAD.

Present investigation focused on untargeted serum metabolite profile of patients with coronary artery disease who are under treatment, by GC–MS and comparison was made with healthy individuals' metabolite profile. The main objective was to highlight the differences in the profiles in order to identify signature changes associated with the patients' condition.

2. Materials and methods

2.1. Chemicals and reagents

Methanol, hexane, N, O-bis-trimethyl tri-fluoroacetamide (BSTFA), and acetone were purchased from Merck, Germany. All other chemicals used were of highest purity grade.

2.2. Blood samples

All the procedures to collect blood from the subjects were done according to ethical guidelines. Fresh blood samples were obtained from King Khalid University Hospital. Ethical approval was granted for these experiments by the Institutional Review Board at King Saud University Medical City, with approval number (Research Project No. E-16-1844). Blood samples were collected from patients as well as healthy individuals in serum vacutainers. Serum was collected and stored at −80 °C for further investigations. Collected samples were processed for metabolomics analysis by GC–MS.

A total 76 samples were analyzed in the present investigation. 71 samples were from patients and five samples were from healthy individuals for comparison.

2.3. Sample preparation for GC–MS analysis

Serum samples were thawed and vortexed at room temperature. In 100 μL of plasma sample, 300 μL of methanol and 100 μL of distilled water were added and the mixture was vortexed properly for 2 min. The total mixture was centrifuged at 10,000 rpm for 10 min at 4 °C. 200-μl of supernatant sample was transferred to GC vial and evaporated under nitrogen stream. Into this vial, 100 μL of methoxylamine HCl/pyridine (20 mg/mL) was added and vortexed the mixture for 5 min. The sample vial was kept at room temperature overnight to complete the methoxymation reaction. After overnight incubation at room temperature, 100 μL N, O-bis-trimethyl tri-fluoroacetamide (BSTFA) was added and vortex for 5 min and kept for 30 min at 50 °C to complete the derivatization procedure. Finally 100 μL of hexane was added in the mixture. This final mixture was subjected to qualitative analysis by GC–MS.

2.4. Instrumentation

All the samples were analyzed using Clarus 600 T, Perkin Elmer that was combined with single quadrupole mass spectrometer. Elite 5MS column (30 m × 0.25 mm × 0.25 μm film thickness), were used for the separation and the carrier gas used was ultrapure helium at a flow rate of 1 mL/min. a splitless injector at 20:1 was used at a temperature of 280 °C. The temperature was set initially to 40 °C (held for 2 min), was increased to 150 °C at 5° C min^{-1} (held for 2 min), then increased further to 280 °C at 10 °C min^{-1} for 2 min. The MS ion source temperature was 220 °C and inlet line temperature at 240 °C. The scan range was set at 40 to 600 mass ranges at 70 ev electron energy and the solvent delay of 4 min. Finally, unknown compounds were identified by comparing the spectra with that of the NIST 2005 (National Institute of Standard and Technology library) and Wiley 2006 library. The total time required for analyzing a single sample was 41 min.

2.5. Statistical analysis

Statistical analysis of data was done using JASP statistical software (JASP Team (2019). JASP (Version 0.10.2)[Computer software]. The statistics included descriptive statistics, student T test, Pearson's correlation and Principal Component Analysis (PCA). PCA included parallel analysis with orthogonal, varimax rotation method. Numerical values from AUCs were used for the analysis.

3. Results

GC–MS investigation identified 17 metabolites in serum samples (Table 1). The peaks were identified by NIST and Wiley library and the numerical data was collected in form of AUCs. Statistical analyses were done using this data.

Descriptive statistical analysis revealed that the data from control samples was found to be normally distributed (Shapiro-Wilk test) among all the samples for all the metabolites except ribitol (Table 2), however it was not found to be normally distributed in patient samples with Acetohydroxamic acid as an exception (Table 3). Boxplots in Figs. 1 and 2 show data distribution among control and patient samples respectively.

Student's T-test reveals that AUCs of patient group's alanine, malonic acid, ribitol, D-glucose, mannose ($P < 0.001$), acetohydroxamic acid, N-carboxyglycine, and aminobutyrate ($P < 0.05$) are significantly smaller than in control group (Table 4). The differences in AUCs of other metabolites were not found to be significant (Table 4).

Pearson Correlation Analysis was performed to seek out correlations among different metabolites detected in serum samples from healthy individuals and patients. Table 5 shows the correlation matrix obtained after the analysis of the metabolite data from different metabolites. It indicates that correlation exist among almost all the metabolites. However, some of them show highly significant

Table 1
Metabolites detected by GC–MS (matches within GC–MS libraries) in serum samples from healthy individuals and coronary artery disease patients.

S. No.	Metabolites
1	Alanine
2	Valine
3	lactic acid
4	Acetohydroxamic acid
5	benzenepropanoic acid
6	N-carboxy glycine
7	Gamma lactone
8	Aminobutyrate
9	Malonic acid
10	Ribitol
11	D-glucose
12	Mannose
13	D-sorbitol
14	Hexadecanoic
15	Trans-9-octadecanoic acid
16	Octadecanoic acid
17	Heptadecanoic acid

Table 2
Descriptive statistics of metabolites detected in control serum samples.

	Alanine	Valine	Lactic acid	Acetohydroxamic acid	Benzene propanoic acid	N-carboxy glycine	Gamma lactone	Aminobutyrate	Malonic acid	Ribitol	D-glucose	Mannose	D-sorbitol	Hexadecanoic	Trans-9-octadecanoic acid	Octadecanoica cid	Heptadecanoica cid
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Mean AUC	40469.000	71234.600	96466.200	3014.000	54430.400	49777.200	599.200	114991.800	4.199e+6	121718.600	7.166e+6	1.365e+6	732504.000	41821.000	347485.600	11742.200	5702.400
Std. Error of Mean	7429.759	25449.302	39849.121	794.683	9528.149	5776.830	255.298	16129.113	388601.163	49798.281	1.096e+6	96527.731	294740.344	19335.912	126592.726	3353.931	1286.016
Median	45154.000	92066.000	50394.000	2416.000	60616.000	51096.000	287.000	124582.000	4.281e+6	71198.000	6.610e+6	1.481e+6	1.028e+6	20831.000	206622.000	12891.000	5679.000
Std. Deviation	215842.568				16613.447	56906.370		89105.343 659059.445	1776.965 43236.415	21305.589 283069.940		12917.385 7499.618	570.863 2875.619	36065.792	868938.617	111352.342	2.450e+6
Shapiro-Wilk	0.747	0.880	0.806	0.879	0.773	0.944	0.799	0.838	0.959	0.609	0.788	0.842	0.784	0.616	0.695	0.950	0.985
P-value of Shapiro-Wilk	0.001	0.008	0.736	0.961	0.028	0.308	0.091	0.305	0.047	0.695	0.079	0.160	0.803	<0.001	0.064	0.171	0.059
Minimum	11575.000	203.000	30030.000	1397.000	17486.000	31002.000	198.000	54815.000	3.163e+6	64730.000	5.194e+6	1.028e+6	20062.000	19931.000	179615.000	165.000	2195.000
Maximum	51664.000	126910.000	244896.000	5942.000	70078.000	67213.000	1527.000	143457.000	5.498e+6	320486.000	1.140e+7	1.538e+6	1.322e+6	118867.000	844542.000	20742.000	9885.000

Table 3
Descriptive statistics of metabolites detected in coronary artery disease patients' serum samples.

	Alanine	Valine	Lactic acid	Acetohydroxamic acid	Benzene propanoic acid	N-carboxy glycine	Gamma lactone	Aminobutyrate	Malonic acid	Ribitol	D-glucose	Mannose	D-sorbitol	Hexadecanoic	Trans-9-octadecanoic acid	Octadecanoica cid	Heptadecanoica cid
N	71	71	71	71	71	71	71	71	71	71	71	71	71	71	71	71	71
Mean AUC	14127.831	50981.577	63218.141	1652.127	35760.479	16594.465	1398.437	57965.718	4541.310	7079.127	1.030e+6	122401.239	1.120e+6	39272.225	487055.014	28763.789	6869.521
Std. Error of Mean	1369.521	5352.018	11935.439	133.976	3351.816	2572.510	280.822	5515.977	1654.704	706.974	134987.955	25317.485	243275.069	14119.328	147037.069	11002.108	2214.685
Median	13180.000	53502.000	32223.000	1772.000	45251.000	13626.000	603.000	65295.000	1177.000	5608.000	801606.000	80298.000	105557.000	14189.000	173619.000	7510.000	3553.000
Std. Deviation	213328.924				11539.785 2.050e+6	45096.902		100569.799 118971.570	1128.900 1.239e+6		28242.907 92705.408	21676.357 18661.264	2366.247	46478.449	13942.780	5957.071	1.137e+6
Shapiro-Wilk	0.769	0.851	0.573	0.954	0.855	0.580	0.575	0.891	0.315	0.833	0.692	0.393	0.611	0.271	0.353	0.290	0.272
P-value of Shapiro-Wilk	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.011	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Minimum	22.000	38.000	29.000	24.000	15.000	26.000	31.000	25.000	39.000	32.000	158.000	174.000	123.000	155.000	1600.000	71.000	127.000
Maximum	80619.000	133606.000	695536.000	4657.000	81795.000	159100.000	11936.000	244594.000	88062.000	30205.000	8.002e+6	1.751e+6	8.702e+6	832394.000	7.550e+6	558134.000	153099.000

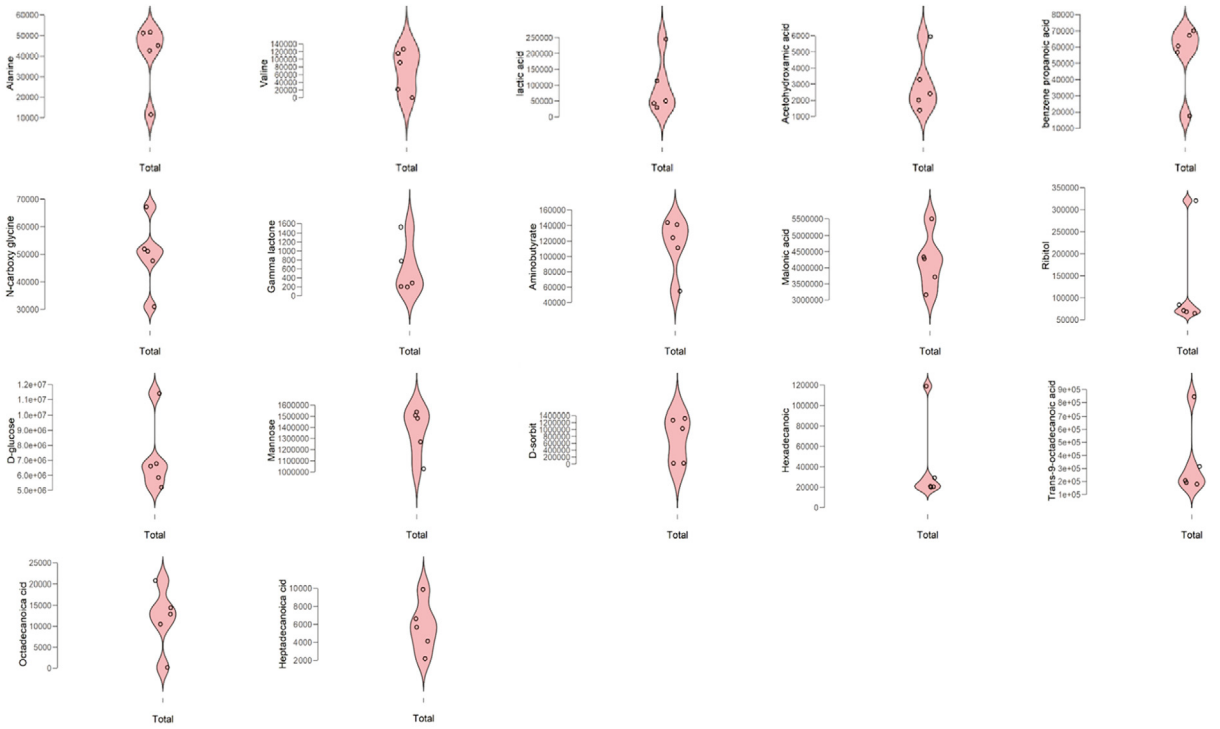

Fig. 1. Boxplots showing distribution of metabolites data among control serum samples.

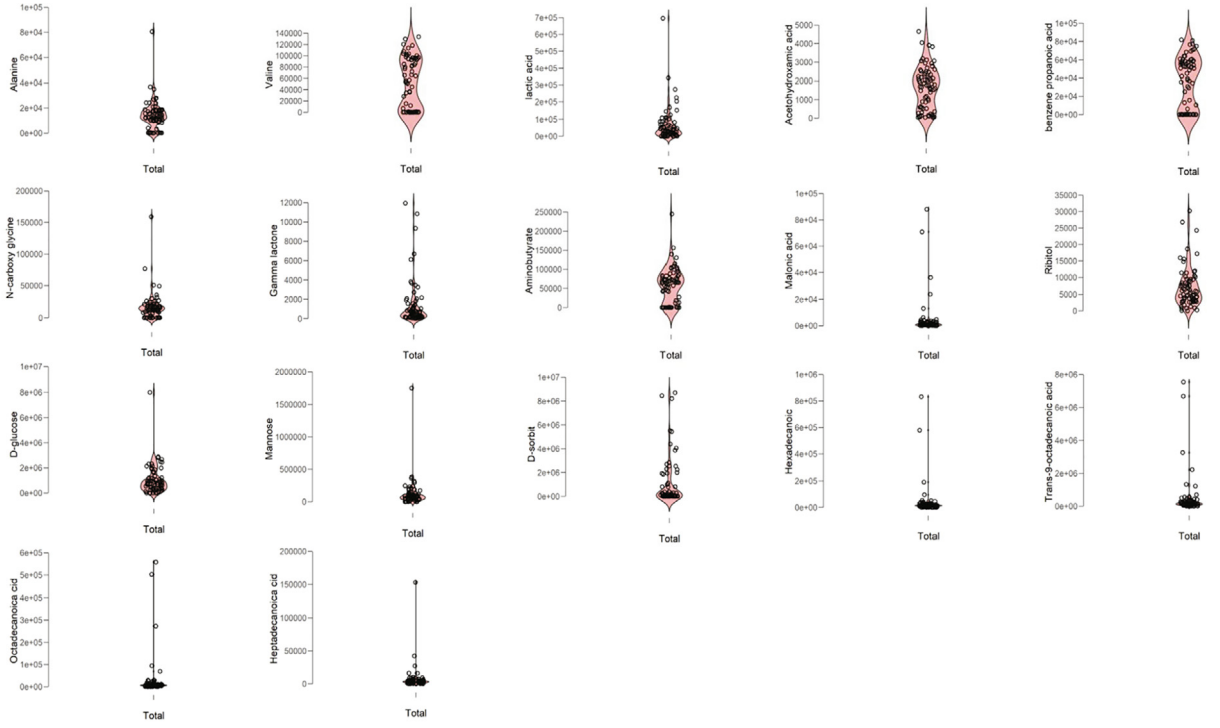

Fig. 2. Boxplots showing distribution of metabolites data among coronary artery disease patients' serum samples.

($P < 0.001$) correlation. These include alanine showing high correlation with melonicacid, ribitol, D-glucose, and mannose; valine with benzenepropanoic acid, and aminobutyrate; acetohydroxamic acid with N-carboxy glycine, aminobutyrate, ribitol and D-glucose; benzenepropanoic acid with aminobutyrate; N-carboxyglycine with D-glucose, and mannose; gamma lactone with D-sorbit; malonic acid with ribitol, D-glucose, and mannose; ribitol with D-glucose, and mannose; D-glucose with mannose; hexadecanoic acid with *trans*-9-octadecanoic acid, octadecanoic acid (Stearic acid), and heptadecanoic acid; *trans*-9-octadecanoic acid with octadecanoic; octadecanoic with heptadecanoic acid. It clearly appears that most of the metabolites belonging to same

Table 4
Shows outcomes of T-test analysis of metabolites data comparing individual metabolites in control samples with coronary artery disease patient samples.

Metabolites	t	df	p
Alanine	4.796	74.000	<0.001
Valine	0.955	74.000	0.342
lactic acid	0.719	74.000	0.475
Acetohydroxamic acid	2.509	74.000	0.014
benzenepropanoic acid	1.446	74.000	0.153
N-carboxyglycine	3.368	74.000	0.001
Gamma lactone	−0.749	74.000	0.456
Aminobutyrate	2.681	74.000	0.009
Malonic acid	44.773	74.000	<0.001
Ribitol	9.339	74.000	<0.001
D-glucose	10.658	74.000	<0.001
Mannose	12.583	74.000	<0.001
D-sorbitol	−0.419	74.000	0.676
Hexadecanoic acid	0.047	74.000	0.962
Trans-9-octadecanoic acid	−0.250	74.000	0.803
Octadecanoic acid	−0.408	74.000	0.685
Heptadecanoic acid	−0.139	74.000	0.890

Note. Student's *t*-test.

class of chemicals are highly correlated with each other for example fatty acids and sugars indicated above. Principal component analysis (PCA) reveals more about different variables having correlations with one another.

Principal Component Analysis was done to identify the cluster of metabolites showing similar trends. The Analysis of serum metabolites data found three components out of 17 metabolites; RC1 (Acetohydroxamic acid, alanine, D-glucose, malonic acid, mannose, N-carboxy glycine and ribitol), RC2 (Heptadecanoic acid, hexadecanoic acid, octadecanoic acid and Trans-9-octadecanoic acid), RC3 (Aminobutyrate, D-sorbit, gamma lactone, valine, benzene propanoic acid and lactic acid) (Table 6). All the fatty acids constituted RC2 and appear to be strictly correlating with one another as became apparent in Pearson correlation analysis as well. Fig. 3 shows the path diagram indicating interactions among different components of the metabolites data. Fig. 4, a scree plot, is showing eigenvalues of the different components. The horizontal line, in Fig. 4, at eigenvalue 1 indicates "Kaiser rule" criteria.

4. Discussion

Metabolites identification and quantification as an endpoint parameter has served as a significant tool in diagnostics, pharmacology, toxicology and therapeutics, etc. However, investigation of single metabolite has limited scope and scarce chances of finding novel biomarkers of diseases and toxicant exposures. Metabolomics profiling of the metabolites on the other side comes with several advantages including identification of several (up to hundreds) metabolites in fewer experiments, increased chances of finding novel biomarkers of diseases and exposures and possibility of using the metabolomics profile as a unique fingerprint associated with a particular condition.

Present investigation involved metabolomics profiling of serum samples from patients with coronary artery disease and healthy subjects. GC–MS technique, which was selected over the more robust NMR due to its higher sensitivity, identified 17 major peaks. Library matches yielded 17 metabolites listed in Table 1. Human serum is reported to contain larger number of metabolites than identified in the present study, but the number of detected metabolites depends on adopted methods and a combination of several methods may be needed to achieve that.

Comparison of patient sample profile with control samples found that eight metabolites exhibit significant differences in AUCs. These include alanine, malonic acid, ribitol, D-glucose, mannose, acetohydroxamic acid, N-carboxyglycine, and aminobutyrate. The AUCs of these eight metabolites were found to be significantly smaller in patient samples. Interestingly, Principal Component Analysis reveals that seven out of these eight metabolites accumulate in principal component RC1, leaving aminobutyrate an odd one which is in principal component RC2. It indicates that the metabolites in RC1 probably are playing important role associated with coronary artery disease (CAD) progression. Acetohydroxamic acid is known to be a synthetic drug that is a urease inhibitor in plants and bacteria, and is used as adjunctive therapy in urinary tract infections (Griffith and Musher, 1975; Lake and Brown, 1985). However, the presence of the acetohydroxamic acid is not fully understood in the present investigation as no patient reported taking this drug, similar finding was reported by Titan et al. in chronic kidney disease patients (Titan et al. 2019) where the same metabolite was detected in the sample of patients who were not on a therapy with acetohydroxamic. More investigations are needed to understand presence of acetohydroxamic acid in human metabolome. Analysis of the data by MetaboAnalyst 4.0 (data not given) reveal that the identified metabolites are associated with 29 different metabolic pathways, indicating pyruvate metabolism pathways being the most impactful among them. Other lesser impactful pathways included alanine, aspartate and glutamate metabolism; tyrosine metabolism; fatty acid metabolism; starch and sucrose metabolism; fructose and mannose metabolism; taurine and hypotaurine metabolism; beta-alanine metabolism; valine, leucine and isoleucine biosynthesis, and phenylalanine, tyrosine and tryptophan biosynthesis.

All the four fatty acids detected were clustered in principal component RC2. In T-test analysis, it was observed that AUCs of *trans*-9-octadecanoic acid, octadecanoic acid and heptadecanoic acid were found to be increased in patient samples but it was not found to be statistically significant. However, this increase is expected in coronary heart diseases and researchers has reported an increase in total saturated fatty acids, including stearic acid/octadecanoic acid in plasma of coronary heart diseases patients (Wang et al. 2003). D-sorbitol, gamma-lactone and lactic acid, which were clustered together in principal component RC3 with other three metabolites, also appear to be important as there AUCs were noted to be larger when compared with control samples, but found to be statistically insignificant.

All the data from metabolites analysis was in form of AUCs which was used for the statistical analysis. Similar approaches has been used by researches elsewhere in the past (Huan et al., 2016; Sato et al., 2019). Previously published reports elsewhere and the findings of the present investigation strongly indicate that using AUCs of metabolites' peaks can be a cost effective approach in comparison to quantitative analyses.

An untargeted approach for GC–MS analysis and found eight metabolites that are showing significant variation in AUCs in comparison to control samples. These findings highlight these metabolites (alanine, malonic acid, ribitol, D-glucose, mannose, acetohydroxamic acid, N-carboxyglycine, and aminobutyrate) for further investigation in case of the CAD diagnosis and its treatment. Based on principal component analysis fatty acids including Trans-9-octadecanoic acid, heptadecanoic acid and octadecanoic acid are also important in association with CAD condition.

Author contributions

WQ contributed in design of experiments, analyzed the data and manuscript writing. SA obtained the samples, contributed to planning and design of the study and manuscript writing. SR contributed to design of experiments, GC–MS analysis of samples, data collection and manuscript writing. NA contributed to experimental

Table 5
Correlation among different metabolites (Pearson's correlation) detected in serum samples of healthy individuals and coronary artery disease patients.

		Alanine	Valine	Lactic acid	Acetohydroxamic acid	Benzene propanoic acid	N-carboxy glycine	Gamma lactone	Aminobutyrate	Malonic acid	Ribitol	D-glucose	Mannose	D-sorbit	Hexadecanoic	Trans-9-octadecanoic acid	Octadecanoica cid	Heptadecanoica cid
Alanine	Pearson's r	–																
	p-value	–																
Valine	Pearson's r	−0.049	–															
	p-value	0.675	–															
lactic acid	Pearson's r	0.101	−0.094	–														
	p-value	0.385	0.421	–														
Acetohydroxamic acid	Pearson's r	0.304	0.151	0.138	–													
	p-value	0.008	0.194	0.234	–													
benzene propanoic acid	Pearson's r	−0.037	0.820	−0.153	0.259	–												
	p-value	0.749	<0.001	0.186	0.024	–												
N-carboxy glycine	Pearson's r	0.279	0.106	0.123	0.398	0.173	–											
	p-value	0.015	0.364	0.291	<0.001	0.134	–											
Gamma lactone	Pearson's r	−0.043	−0.096	0.117	−0.008	−0.110	−0.066	–										
	p-value	0.715	0.411	0.316	0.945	0.346	0.569	–										
Aminobutyrate	Pearson's r	0.176	0.640	−0.145	0.438	0.758	0.202	−0.128	–									
	p-value	0.129	<0.001	0.210	<0.001	<0.001	0.080	0.271	–									
Malonic acid	Pearson's r	0.482	0.097	0.106	0.330	0.135	0.367	−0.093	0.261	–								
	p-value	<0.001	0.405	0.363	0.004	0.246	0.001	0.426	0.023	–								
Ribitol	Pearson's r	0.495	−0.072	0.212	0.481	−0.010	0.311	−0.026	0.132	0.825	–							
	p-value	<0.001	0.534	0.066	<0.001	0.930	0.006	0.823	0.255	<0.001	–							
D-glucose	Pearson's r	0.456	0.036	0.309	0.405	0.068	0.390	0.222	0.202	0.816	0.803	–						
	p-value	<0.001	0.756	0.007	<0.001	0.557	<0.001	0.054	0.080	<0.001	<0.001	–						
Mannose	Pearson's r	0.438	0.051	0.256	0.309	0.066	0.413	0.219	0.190	0.831	0.673	0.937	–					
	p-value	<0.001	0.663	0.026	0.007	0.569	<0.001	0.057	0.101	<0.001	<0.001	<0.001	–					
D-sorbitol	Pearson's r	−0.027	−0.095	0.321	0.083	−0.080	0.072	0.490	−0.166	−0.048	0.035	0.245	0.218	–				
	p-value	0.819	0.415	0.005	0.478	0.493	0.535	<0.001	0.152	0.678	0.766	0.033	0.059	–				
Hexadecanoic	Pearson's r	0.121	−0.211	−0.083	−0.093	−0.236	−0.008	0.063	−0.187	0.019	0.084	0.013	0.006	−0.045	–			
	p-value	0.297	0.067	0.478	0.426	0.040	0.948	0.587	0.105	0.869	0.468	0.910	0.956	0.698	–			
Trans-9-octadecanoic acid	Pearson's r	0.136	−0.187	−0.095	−0.140	−0.238	−0.058	0.022	−0.178	−0.021	0.030	−0.051	−0.052	−0.102	0.914	–		
	p-value	0.242	0.106	0.415	0.228	0.038	0.616	0.849	0.124	0.860	0.796	0.663	0.657	0.382	<0.001	–		
Octadecanoica cid	Pearson's r	0.084	−0.169	−0.105	−0.179	−0.230	−0.062	0.041	−0.191	−0.050	−0.029	−0.073	−0.052	−0.076	0.924	0.972	–	
	p-value	0.472	0.144	0.368	0.123	0.045	0.597	0.725	0.098	0.670	0.800	0.532	0.657	0.517	<0.001	<0.001	–	
Heptadecanoica cid	Pearson's r	0.184	−0.157	−0.076	−0.055	−0.169	0.021	0.084	−0.099	−0.020	0.006	−0.002	0.015	−0.059	0.887	0.719	0.738	–
	p-value	0.112	0.176	0.515	0.637	0.144	0.859	0.471	0.393	0.862	0.958	0.987	0.898	0.615	<0.001	<0.001	<0.001	–

Table 6
Principal Component Analysis.

Component Loadings				
	RC 1	RC 2	RC 3	Uniqueness
Acetohydroxamic acid	0.574	.	.	0.603
Alanine	0.611	.	.	0.591
Aminobutyrate	.	.	0.789	0.231
D-glucose	0.931	.	.	0.101
D-sorbitol	.	.	−0.467	0.724
Gamma lactone	.	.	−0.404	0.829
Heptadecanoic acid	.	0.870	.	0.242
Hexadecanoic acid	.	0.971	.	0.050
Malonic acid	0.879	.	.	0.225
Mannose	0.891	.	.	0.180
N-carboxyglycine	0.540	.	.	0.694
Octadecanoic acid	.	0.952	.	0.089
Ribitol	0.855	.	.	0.253
Trans-9-octadecanoic acid	.	0.951	.	0.095
Valine	.	.	0.781	0.333
benzene propanoic acid	.	.	0.829	0.222
lactic acid	.	.	−0.423	0.710

Component Correlations			
	RC 1	RC 2	RC 3
RC 1	1.000	.	.
RC 2	0.000	1.000	.
RC 3	0.000	0.000	1.000

Chi-squared Test			
	Value	df	p
Model	341.680	88	<0.001

Note. Applied rotation method is varimax.

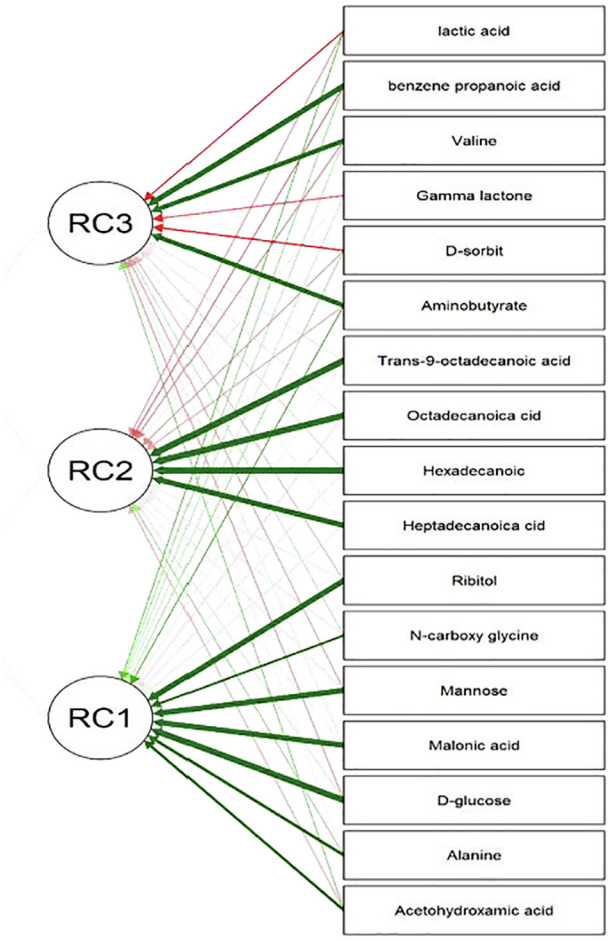

Fig. 3. Path Diagram showing different components obtained after Principal Component Analysis (PCA) of the data from 17 metabolites identified in different serum samples.

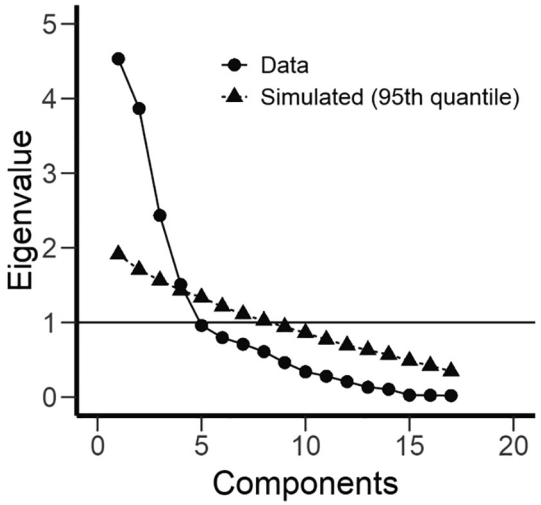

Fig. 4. Showing eigenvalues of different Principal Components.

part, data analysis and manuscript writing. MA contributed to planning of the study and experiments, data collection, execution of experiments and manuscript writing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

CARDIoGRAMplusC4D Consortium, Deloukas, P., Kanoni, S., Willenborg, C., Farrall, M., Assimes, T.L., Thompson, J.R., Ingelsson, E., Saleheen, D., Erdmann, J., Goldstein, B.A., Stirrups, K., König, I.R., Cazier, J.B., Johansson, A., Hall, A.S., Lee, J. Y., Willer, C.J., Chambers, J.C., Esko, T., et al., 2013. Large-scale association analysis identifies new risk loci for coronary artery disease. Nature Gen. 45(1), 25–33. https://doi.org/10.1038/ng.2480.

Gottdiener, J.S., Arnold, A.M., Aurigemma, G.P., Polak, J.F., Tracy, R.P., Kitzman, D.W., Gardin, J.M., Rutledge, J.E., Boineau, R.C., 2000. Predictors of congestive heart failure in the elderly: the Cardiovascular Health Study. J. Am. Coll. Cardiol. 35 (6), 1628–1637. https://doi.org/10.1016/s0735-1097(00)00582-9.

Griffith, D.P., Musher, D.M., 1975. Acetohydroxamic acid. Potential use in urinary infection caused by urea-splitting bacteria. Urology 05 (3), 299–302. https://doi.org/10.1016/0090-4295(75)90142-9.

Huan, T., Troyer, D.A., Li, L., 2016. Metabolite Analysis and Histology on the Exact Same Tissue: Comprehensive Metabolomic Profiling and Metabolic Classification of Prostate Cancer. Sci. Rep. 6, 32272. https://doi.org/10.1038/srep32272.

Jensen, M.K., Bertoia, M.L., Cahill, L.E., Agarwal, I., Rimm, E.B., Mukamal, K.J., 2014. Novel metabolic biomarkers of cardiovascular disease. Nature reviews. Endocrinology 10 (11), 659–672. https://doi.org/10.1038/nrendo.2014.155.

Joseloff, E., Sha, W., Bell, S.C., Wetmore, D.R., Lawton, K.A., Milburn, M.V., Ryals, J.A., Guo, L., Muhlebach, M.S., 2014. Serum metabolomics indicate altered cellular energy metabolism in children with cystic fibrosis. Pediatr. Pulmonol. 49 (5), 463–472. https://doi.org/10.1002/ppul.22859.

Koeth, R.A., Wang, Z., Levison, B.S., Buffa, J.A., Org, E., Sheehy, B.T., Britt, E.B., Fu, X., Wu, Y., Li, L., Smith, J.D., DiDonato, J.A., Chen, J., Li, H., Wu, G.D., Lewis, J.D., Warrier, M., Brown, J.M., Krauss, R.M., Tang, W.H., Hazen, S.L., 2013. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat. Med. 19 (5), 576–585. https://doi.org/10.1038/nm.3145.

Lake, K.D., Brown, D.C., 1985. New drug therapy for kidney stones: a review of cellulose sodium phosphate, acetohydroxamic acid, and potassium citrate. Drug Intell. Clin. Pharm. 19 (7–8), 530–539. https://doi.org/10.1177/106002808501900705.

Lanznaster, D., de Assis, D.R., Corcia, P., Pradat, P.F., Blasco, H., 2018. Metabolomics Biomarkers: A Strategy Toward Therapeutics Improvement in ALS. Front. Neurol. 9, 1126. https://doi.org/10.3389/fneur.2018.01126.

Lau, C.E., Siskos, A.P., Maitre, L., Robinson, O., Athersuch, T.J., Want, E.J., Urquiza, J., Casas, M., Vafeiadi, M., Roumeliotaki, T., McEachan, R., Azad, R., Haug, L.S., Meltzer, H.M., Andrusaityte, S., Petraviciene, I., Grazuleviciene, R., Thomsen, C., Wright, J., Slama, R., Coen, M., 2018. Determinants of the urinary and serum metabolome in children from six European populations. BMC Med. 16 (1), 202. https://doi.org/10.1186/s12916-018-1190-8.

Li, Y., Zhang, D., He, Y., Chen, C., Song, C., Zhao, Y., Bai, Y., Wang, Y., Pu, J., Chen, J., Yang, Y., Dou, K., 2017. Investigation of novel metabolites potentially involved in the pathogenesis of coronary heart disease using a UHPLC-QTOF/MS-based metabolomics approach. Sci. Rep. 7 (1), 15357. https://doi.org/10.1038/s41598-017-15737-3.

Mente, A., Chalcraft, K., Ak, H., Davis, A.D., Lonn, E., Miller, R., Potter, M.A., Yusuf, S., Anand, S.S., McQueen, M.J., 2015. The Relationship Between Trimethylamine-N-Oxide and Prevalent Cardiovascular Disease in a Multiethnic Population Living in Canada. Can. J. Cardiol. 31 (9), 1189–1194. https://doi.org/10.1016/j.cjca.2015.06.016.

Patel, S., Ahmed, S., 2015. Emerging field of metabolomics: big promise for cancer biomarker identification and drug discovery. J. Pharm. Biomed. Anal. 107, 63–74. https://doi.org/10.1016/j.jpba.2014.12.020.

Psychogios, N., Hau, D.D., Peng, J., Guo, A.C., Mandal, R., Bouatra, S., Sinelnikov, I., Krishnamurthy, R., Eisner, R., Gautam, B., Young, N., Xia, J., Knox, C., Dong, E., Huang, P., Hollander, Z., Pedersen, T.L., Smith, S.R., Bamforth, F., Greiner, R., Wishart, D.S., 2011. The human serum metabolome. PloS one 6, (2). https://doi.org/10.1371/journal.pone.0016957 e16957.

Rachakonda, V., Gabbert, C., Raina, A., Bell, L.N., Cooper, S., Malik, S., Behari, J., 2014. Serum metabolomic profiling in acute alcoholic hepatitis identifies multiple dysregulated pathways. PLoS ONE 9, (12). https://doi.org/10.1371/journal.pone.0113860 e113860.

Rzeznik, M., Triba, M.N., Levy, P., Jungo, S., Botosoa, E., Duchemann, B., Le Moyec, L., Bernaudin, J.F., Savarin, P., Guez, D., 2017. Identification of a discriminative metabolomic fingerprint of potential clinical relevance in saliva of patients with periodontitis using 1H nuclear magnetic resonance (NMR) spectroscopy. PLoS ONE 12, (8). https://doi.org/10.1371/journal.pone.0182767 e0182767.

Sato, T., Kawasaki, Y., Maekawa, M., Takasaki, S., Saigusa, D., Ota, H., Shimada, S., Yamashita, S., Mitsuzuka, K., Yamaguchi, H., Ito, A., Kinoshita, K., Koshiba, S., Mano, N., Arai, Y., 2019. Value of global metabolomics in association with diagnosis and clinicopathological factors of renal cell carcinoma. Int. J. Cancer 145 (2), 484–493. https://doi.org/10.1002/ijc.32115.

Spratlin, J.L., Serkova, N.J., Eckhardt, S.G., 2009. Clinical applications of metabolomics in oncology: a review. Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 15 (2), 431–440. https://doi.org/10.1158/1078-0432.CCR-08-1059.

Stander, Z., Luies, L., Mienie, L.J., Keane, K.M., Howatson, G., Clifford, T., Stevenson, E. J., Loots, D.T., 2018. The altered human serum metabolome induced by a marathon. Metabol. Off. J. Metabol. Soc. 14 (11), 150. https://doi.org/10.1007/s11306-018-1447-4.

Steuer, A.E., Brockbals, L., Kraemer, T., 2019. Metabolomic Strategies in Biomarker Research-New Approach for Indirect Identification of Drug Consumption and Sample Manipulation in Clinical and Forensic Toxicology?. Front. Chem. 7, 319. https://doi.org/10.3389/fchem.2019.00319.

Titan, S.M., Venturini, G., Padilha, K., Goulart, A.C., Lotufo, P.A., Bensenor, I.J., Krieger, J.E., Thadhani, R.I., Rhee, E.P., Pereira, A.C., 2019. Metabolomics biomarkers and the risk of overall mortality and ESRD in CKD: Results from the Progredir Cohort. PLoS ONE 14, (3). https://doi.org/10.1371/journal.pone.0213764 e0213764.

Wang, L., Folsom, A.R., Eckfeldt, J.H., 2003. Plasma fatty acid composition and incidence of coronary heart disease in middle aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Nutrit. Metabol. Cardiovasc. Dis. NMCD 13 (5), 256–266. https://doi.org/10.1016/s0939-4753(03)80029-7.