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.

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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 (Joseff 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 (Consolidation of, 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...
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 acetonitrile 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 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 vortexed 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 MS column (30 m × 0.25 mm × 0.25 μm film thickness), were used for the separation and the carrier gas used was ultra-pure 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

| S. No. | Metabolites                  |
|-------|------------------------------|
| 1     | Alanine                      |
| 2     | Valine                       |
| 3     | Lactic acid                  |
| 4     | Acetohydroxamic acid         |
| 5     | Benzenepropanoic acid        |
| 6     | N-carboxyglycine             |
| 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 3
Descriptive statistics of metabolites detected in control serum samples.

| Alanine | Valine | Lactic acid | AcetoIhydroxamic acid | Benzene | N-carboxypropanoic acid | Gamma lactone | Aminobutyrate | Malonic acid | Ribitol | D-glucose | Mannose | D-sorbitol | Hexadecanoic acid | Trans-9-octadecanoic acid | Octadecanoic acid | Heptadecanoic acid |
|---------|--------|-------------|-----------------------|---------|-------------------------|---------------|---------------|--------------|---------|-----------|---------|-----------|------------------|-----------------------|----------------|-------------------|
| N       | 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 | 11491.800 | 4.199e+6 | 12178.600 | 7.166e-6 | 1.365e+6 | 735204.000 | 41821.000 | 347485.600 | 11742.200 |
| Std. Error of Mean | 1369.521 | 5352.018 | 11935.439 | 133.976 | 3351.816 | 2572.510 | 280.822 | 12458.000 | 4.281e+6 | 7118.900 | 6.510e-6 | 1.481e+6 | 20831.000 | 206622.000 | 12691.000 |
| Median | 7429.719 | 25449.302 | 39840.121 | 794.683 | 9528.149 | 5776.830 | 255.298 | 16125.113 | 388601.163 | 49708.281 | 1.096e-6 | 95627.731 | 294740.344 | 19335.912 | 126592.726 |
| 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 | 0.088 | 0.961 | 0.736 | 0.091 | 0.305 | 0.047 | 0.695 | 0.079 | 0.160 | 0.803 | <0.001 | 0.064 | 0.171 | 0.095 |
| Minimum | 51575.000 | 203.000 | 30030.000 | 1397.000 | 17486.000 | 31002.000 | 198.000 | 54815.000 | 3.163e+6 | 64730.000 | 5.194e-6 | 20062.000 | 19931.000 | 179615.000 | 165.000 | 2195.000 |
| Maximum | 51644.000 | 126910.000 | 244896.000 | 5942.000 | 70078.000 | 67213.000 | 1527.000 | 141457.000 | 5.498e+6 | 320486.000 | 1.140e+7 | 1.322e+6 | 118867.000 | 844542.000 | 20742.000 | 9885.000 |

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

| Alanine | Valine | Lactic acid | AcetoIhydroxamic acid | Benzene | N-carboxypropanoic acid | Gamma lactone | Aminobutyrate | Malonic acid | Ribitol | D-glucose | Mannose | D-sorbitol | Hexadecanoic acid | Trans-9-octadecanoic acid | Octadecanoic acid | Heptadecanoic acid |
|---------|--------|-------------|-----------------------|---------|-------------------------|---------------|---------------|--------------|---------|-----------|---------|-----------|------------------|-----------------------|----------------|-------------------|
| N       | 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 | 11491.800 | 4.199e+6 | 12178.600 | 7.166e-6 | 1.365e+6 | 735204.000 | 41821.000 | 347485.600 | 11742.200 |
| Std. Error of Mean | 1369.521 | 5352.018 | 11935.439 | 133.976 | 3351.816 | 2572.510 | 280.822 | 12458.000 | 4.281e+6 | 7118.900 | 6.510e-6 | 1.481e+6 | 20831.000 | 206622.000 | 12691.000 |
| Median | 7429.719 | 25449.302 | 39840.121 | 794.683 | 9528.149 | 5776.830 | 255.298 | 16125.113 | 388601.163 | 49708.281 | 1.096e-6 | 95627.731 | 294740.344 | 19335.912 | 126592.726 |
| 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 | 0.088 | 0.961 | 0.736 | 0.091 | 0.305 | 0.047 | 0.695 | 0.079 | 0.160 | 0.803 | <0.001 | 0.064 | 0.171 | 0.095 |
| Minimum | 51575.000 | 203.000 | 30030.000 | 1397.000 | 17486.000 | 31002.000 | 198.000 | 54815.000 | 3.163e+6 | 64730.000 | 5.194e-6 | 20062.000 | 19931.000 | 179615.000 | 165.000 | 2195.000 |
| Maximum | 51644.000 | 126910.000 | 244896.000 | 5942.000 | 70078.000 | 67213.000 | 1527.000 | 141457.000 | 5.498e+6 | 320486.000 | 1.140e+7 | 1.322e+6 | 118867.000 | 844542.000 | 20742.000 | 9885.000 |

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(P < 0.001) correlation. These include alanine showing high correlation with melonic acid, 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-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 octadeanoic; octadeanoic with heptadecanoic acid. It clearly appears that most of the metabolites belonging to same
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 Transit-9-octadecanoic acid), and RC3 (Aminobutyrate, D-sorbit, gamma lactone, N-carboxyglycine and ribitol), RC2 (Heptadecanoic acid, hexadecanoic acid, octadecanoic acid and Transit-9-octadecanoic acid), and RC3 (Aminobutyrate, D-sorbit, gamma lactone, N-carboxyglycine and ribitol) (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 acid. 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 less 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 in the past (Huan et al., 2016; Sato et al., 2019) 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 4

| Metabolites     | t     | df   | p    |
|-----------------|-------|------|------|
| Alanine         | 0.476 | 74.000 | 0.001 |
| Valine          | 0.955 | 74.000 | 0.342 |
| lactic acid     | 0.719 | 74.000 | 0.473 |
| 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    | 4.473 | 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.
| Metabolite                | Alanine | Valine | Lactic acid | Acetohydroxamic acid | Benzene propanoic acid | N-carboxy glycine | Gamma lactone | Aminobutyrate | Malonic acid | Ribitol | D-glucose | Mannose | D-sorbit | Hexadecanoic acid | Trans-9-octadecanoic acid | Octadecanoic acid | Heptadecanoic acid |
|--------------------------|---------|--------|-------------|----------------------|------------------------|------------------|---------------|---------------|--------------|----------|-----------|---------|---------|------------------|---------------------|----------------|----------------------|
| Alanine                  | Pearson’s r | –      | –           | –                    | –                      | –                | –             | –             | –            | –        | –         | –       | –       | –                | –                   | –              | –                    |
| Valine                   | Pearson’s r | –      | –           | –                    | –                      | –                | –             | –             | –            | –        | –         | –       | –       | –                | –                   | –              | –                    |
| Lactic acid              | Pearson’s r | 0.675  | –           | –                    | –                      | –                | –             | –             | –            | –        | –         | –       | –       | –                | –                   | –              | –                    |
| Acetohydroxamic acid     | Pearson’s r | 0.304  | 0.131       | 0.138                | –                      | –                | –             | –             | –            | –        | –         | –       | –       | –                | –                   | –              | –                    |
| Benzene propanoic acid   | Pearson’s r | 0.008  | 0.194       | 0.234                | –                      | –                | –             | –             | –            | –        | –         | –       | –       | –                | –                   | –              | –                    |
| N-carboxy glycine        | Pearson’s r | 0.749  | <0.001      | 0.186                | 0.024                  | –                | –             | –             | –            | –        | –         | –       | –       | –                | –                   | –              | –                    |
| Gamma lactone            | Pearson’s r | 0.279  | 0.106       | 0.123                | 0.398                  | 0.173            | –             | –             | –            | –        | –         | –       | –       | –                | –                   | –              | –                    |
| Aminobutyrate            | Pearson’s r | 0.015  | 0.364       | 0.291                | <0.001                 | 0.134            | –             | –             | –            | –        | –         | –       | –       | –                | –                   | –              | –                    |
| Malonic acid             | Pearson’s r | 0.715  | 0.411       | 0.316                | 0.945                  | 0.346            | 0.569         | –             | –            | –        | –         | –       | –       | –                | –                   | –              | –                    |
| Ribitol                  | Pearson’s r | 0.129  | <0.001      | 0.210                | <0.001                 | 0.080            | 0.271         | –             | –            | –        | –         | –       | –       | –                | –                   | –              | –                    |
| D-glucose                | Pearson’s r | 0.482  | 0.097       | 0.106                | 0.310                  | 0.135            | 0.567         | –             | –            | –        | –         | –       | –       | –                | –                   | –              | –                    |
| Mannose                  | Pearson’s r | 0.495  | 0.072       | 0.212                | 0.481                  | <0.010           | 0.311         | 0.026         | 0.132        | 0.825    | –         | –       | –       | –                | –                   | –              | –                    |
| D-sorbitol               | Pearson’s r | <0.001 | 0.405       | 0.363                | 0.004                  | 0.246            | 0.001         | 0.426         | 0.023        | –        | –         | –       | –       | –                | –                   | –              | –                    |
| Hexadecanoic acid        | Pearson’s r | 0.012  | 0.211       | 0.083                | 0.083                  | 0.081            | 0.304         | 0.051         | 0.187        | 0.816    | 0.801     | –       | –       | –                | –                   | –              | –                    |
| Trans-9-octadecanoic acid| Pearson’s r | 0.081  | 0.415       | 0.005                | 0.478                  | 0.493            | 0.535         | 0.001         | 0.152        | 0.678    | 0.766     | 0.033   | 0.059   | –                | –                   | –              | –                    |
| Octadecanoic acid        | Pearson’s r | 0.121  | –0.211      | –0.083               | –0.093                 | –0.236           | –0.008        | 0.063         | –0.187       | 0.019    | 0.084     | 0.011   | 0.006   | –0.045           | –                   | –              | –                    |
| Heptadecanoic acid       | Pearson’s r | 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            | –                   | –              | –                    |
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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