Plasma fatty acid metabolic profiling and biomarkers of type 2 diabetes mellitus based on GC/MS and PLS-LDA

Lun-Zhao Yi, Jun He, Yi-Zeng Liang, Da-Lin Yuan, Foo-Tim Chau

Abstract Metabolic profiling has increasingly been used as a probe in disease diagnosis and pharmacological analysis. Herein, plasma fatty acid metabolic profiling including non-esterified fatty acid (NEFA) and esterified fatty acid (EFA) was investigated using gas chromatography/mass spectrometry (GC/MS) followed by multivariate statistical analysis. Partial least squares-linear discrimination analysis (PLS-LDA) model was established and validated to pattern discrimination between type 2 diabetic mellitus (DM-2) patients and health controls, and to extract novel biomarker information. Furthermore, the PLS-LDA model visually represented the alterations of NEFA metabolic profiles of diabetic patients with abdominal obesity in the treated process with rosiglitazone. The GC/MS-PLS-LDA analysis allowed comprehensive detection of plasma fatty acid, enabling fatty acid metabolic characterization of DM-2 patients, which included biomarkers different from health controls and dynamic change of NEFA profiles of patients after treated with medicine. This method might be a complement or an alternative to pathogenesis and pharmacodynamics research. © 2006 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Type 2 diabetes mellitus; Fatty acid profiling; Biomarker; Partial least squares-linear discrimination analysis (PLS-LDA); Rosiglitazone

1. Introduction

Metabolomics has distinct advantages over other omics approaches in efficiently building knowledge of biological status because intermediary metabolism is proximal to phenotype, and because metabolites can be measured quantitatively and comprehensively [1,2]. Based on the multivariate analysis of complex biological profiles, metabolomics has successfully applied to many fields such as plant genotype discrimination [3–5], toxicological screening [6] and disease diagnosis [7–10]. NMR is the most commonly used technique for metabolomics because its advantages of being non-destructive, applicable to intact biomaterials, and accessing intrinsically more information rich in complex-mixture analyses, while it is difficult to accurate qualitative and quantitative analysis of metabolites [11–14]. Gas chromatography/mass spectrometry (GC/MS) is a relatively low cost alternative that provides high separation efficiency to resolve the complex biological mixtures. This technique coupled with pattern recognition has been proved to be a good choice for metabolite profiling of plant genotype [3].

Type 2 diabetes mellitus (DM-2) disease is a typical metabolism disorder disease [15]. There are predictions that DM-2 could increase worldwide to more than 250 million individuals within the next decade or so. Despite this, we still are unclear as to its causes and its optimal treatment. A number of studies about this disease have demonstrated that diabetes mellitus is directly associated with metabolism disorder of lipid, especially fatty acids because they provide an important energy source as nutrients, and also act as signaling molecules in various cellular processes, including insulin secretion and cardiovascular risk [16–19]. Once diabetes develops, non-esterified fatty acid (NEFA) plasma levels show a linear correlation with blood glucose levels and hepatic glucose production, regardless of whether the person is lean or obese [20–22]. Although there are some publications associated with fatty acid and its relationship with type 2 diabetes mellitus, none of these involved comprehensive quantitative analysis of NEFAs and esterified fatty acids (EFA), 3D discrimination model of DM-2 patients and health controls [23].

Thiazolidinediones (TZDs) is a class of drugs which work to lower the resistance to insulin in fat, liver and muscle cells and also, by stopping abnormalities and dysfunctions in β-cells, were proved to have the ability to release total free fatty acids in plasma [24,25]. Fatty acid metabolic profiles of patients’ plasma will have some changes when treated with TZDs [26]. However, it is not known how each NEFA changed and which those changes will not only help us to investigate the alterations of marked metabolites, but also give us some guidance to clinic treatment. Unfortunately, there are not any reports about this research.

In this paper, we employed GC/MS technology in order to study the comprehensive quantitative NEFA and EFA metabolic profiling. Then, principal component analysis (PCA) method was used to overview the distribution of all 123 human plasma samples and try to classify the type 2 diabetic mellitus (DM-2) patients and healthy persons (controls). In order to visual represent the alterations of NEFA metabolic profiles of type 2 diabetic patients treated with rosiglitazone, we
employed the supervised partial squares-linear discrimination analysis (PLS-LDA) method to construct a 3D visual model which has good predictive power. Herein, we show the benefits of the model not only to discriminate the DM-2 and controls, but also visual represent the variances of metabolites when treated with rosiglitazone. Furthermore, the key NEFAs (potential biomarkers) which have the most influence on the separation between sample classes were found out by the help of PLS-LDA model.

2. Materials and methods

2.1. Sample collection

Human plasma samples were collected from 45 healthy adults (age range was 24-78), 78 patients with DM-2 (age range was 26-67). All type 2 diabetic patients were from the Xiangya Hospital of Hunan of China with a fasting plasma glucose concentration above 7.0 mmol L\(^{-1}\). Furthermore, we picked up 10 abdominal obesity patients with DM-2 to be treated with rosiglitazone (six women and four men). Plasma samples of these 10 patients were collected at 0 (n = 10), 20 (n = 5), 40 (n = 7), 90 (n = 10) and 14 weeks (n = 6) after treated with rosiglitazone. All clinical experiments were approved by Xiangya Institutional Human Subjects Committee.

2.2. Solvents and standards

Oleic acid (C18:1n-9, Purity: minimum 99.0%), oleic acid methyl ester (C18:1n-9 methyl ester, Purity: minimum 99.0%), 11-octadecenoic acid (C18:1n-7, Purity: minimum 99.0%), heptadecanoic acid (C17:0, Purity: minimum 99.0%), oleic acid (C18:1n-3, Purity: minimum 99.0%), γ-linolenic acid (C18:3n-6, Purity: minimum 99.0%), linolenic acid (C18:3n-3, Purity: minimum 99.0%), linolenic acid methyl ester (C17:0 methyl ester, Purity: minimum 99.0%) and heptadecanoic acid methyl ester (C17:0 methyl ester, Purity: minimum 99.0%) were purchased from Sigma (St. Louis, MO, USA). The solution 5% \( \text{H}_{2}\text{SO}_4/\text{CH}_3\text{OH} \) was freshly prepared by diluting \( \text{H}_2\text{SO}_4 \) (Purity: minimum 98.0%) by chromatographic grade methanol, and 0.4 M KOH/CH\(_3\)OH was freshly prepared in our laboratory by dissolving a reagent grade KOH in methanol.

2.3. Sample preparation

Each collected blood sample was immediately centrifuged at 3000 \( \times \) g for 10 min and plasma was transferred into a clean Eppendorf tube. The plasma samples were stored at \(-80^\circ\)C until analysis. Aliquots (200 \( \mu \)l) of plasma were spiked with internal standard (I.S.) working solution (25 \( \mu \)l C17:0 and 25 \( \mu \)l C17:0 methyl ester) and 2 ml 0.4 M KOH/CH\(_3\)OH was added, vortex-mixed for 30 s and placed at room temperature for 10 min, and then extracted with 2 ml of hexane twice using a vortex mixer for 30 s. The hexane phase was the EFA methyl esters, and this phase was evaporated to dryness under \( \text{N}_2 \) gas. After that, some anhydrous sodium sulfate were added to remove water and 2 ml 5% \( \text{H}_2\text{SO}_4/\text{CH}_3\text{OH} \) was added to the residual phase of plasma, reacted at 70\(^\circ\)C water bath for 30 min. Then, it was extracted with 2 ml of hexane twice using a vortex mixer for 30 s and the NEFA methyl esters were obtained. Samples were evaporated to dryness under \( \text{N}_2 \) gas. Hexane (100 \( \mu \)l) was added to each tube prior to analysis.

2.4. Gas chromatography–mass spectrometry

All GC–MS analyses were performed by a Shimadzu GC2010A (Kyoto, Japan) gas chromatography instrument coupled to a GCMS-QP2010 mass spectrometer (Compaq-Pro Linear data system, class5K software). In the gas chromatographic system, a DB-WAX capillary column (30 m × 0.25 mm I.D., film thickness 0.25 μm) was used. The GC column temperature program is shown in Table 1.

The total GC run time was 14 min. Inlet temperature was kept at 250\(^\circ\)C. Helium carrier gas was used at a constant flow rate of 1.0 ml min\(^{-1}\). A sample of 1.0 \( \mu \)l was injected, the split ratio of the injector being 1:50. Mass conditions were: ionization voltage, 70 eV; ion source temperature, 200\(^\circ\)C; full scan mode in the 30–450 \( \mu \)m mass ranges with 0.2 s scan velocity.

<table>
<thead>
<tr>
<th>Start time (min)</th>
<th>Rate (°C/min)</th>
<th>End time (min)</th>
<th>Final temperature (°C)</th>
<th>Hold time (min)</th>
</tr>
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</tr>
<tr>
<td>5</td>
<td>6</td>
<td>10</td>
<td>180</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>12</td>
<td>220</td>
<td>2</td>
</tr>
</tbody>
</table>

2.5. Feature selection

The general purpose of feature selection is to find the best combination of features, which provides the best classification result. The irrelevant variables that introduce noise should be eliminated. In this study, the variance weights of individual variables were calculated to obtain the best feature combination to discriminate the DM-2 and control. These weights were the ratio of between-class variance to within-class variance for the training groups. For two classes, subscripts T and C here denote the class of DM-2 patients and the class of health controls, respectively. Then, the weight of the \( j \)th variable \((j = 1, 2, \ldots, 21)\) could be obtained using the following equation:

\[
nc_f r_f / n_f \sum_{i=1}^{n_f} (x_{fj} - x_{j})^2 + nc_t r_t / n_t \sum_{i=1}^{n_t} (x_{tj} - x_{j})^2
\]

\((c \text{ or } c' = 1, 2, \ldots, n, \text{ and } t \text{ or } t' = 1, 2, \ldots, n)\)

Here \( nc = n_f + n_t = n \). Subscripts \( c \) and \( c' \) denote the indices of the samples in class C and T, respectively. Thus, \( x_{ij} \) represents the value of the \( j \)th variable of sample \( c \).

2.6. Principal component analysis (PCA) of the GC–MS data

The autoscaled data of EFA and NEFA were analysed by PCA to establish any ‘groupings’ with respect to type 2 diabetic patients and health controls. A PCA model was constructed using all samples. The scores plot of PC1 versus PC2 was examined for separation or clusters relating to the two groups, DM-2 and control.

2.7. Prediction and application of model by partial squares-linear discrimination analysis (PLS-LDA)

As PCA indicated the presence of DM-2 and health controls separation, data were analysed by PLS-LDA with a view to establish whether the separation between DM-2 and control was significant by prediction of class. PLS-LDA models were constructed to establish the significance of the difference of DM-2 and health controls. For the purpose of cross-validation and due to the small number of samples, 10 validation PLS-LDA models were calculated excluding 25% of the samples per class in each validation model. Class membership was predicted using discrimination plane between two classes. Furthermore, the determined PLS-LDA model was applied to visually represent the variance of NEFA metabolic profiles of type 2 diabetic patients treated with rosiglitazone.

2.8. Finding potential biomarkers

If the first three PLS latent variables (LVs) were extracted to establish the LDA model, the equation of the discrimination plane could be expressed as follows:

\[
\sum_{i=1}^{n_f} t_i x_{i1} + \sum_{i=1}^{n_t} t_i x_{i2} + t_i x_{i3} = c
\]

where \( t_i \) and \( x_i \) are the first three LVs obtained by PLS decomposition, \( x_{i1} \), \( x_{i2} \) and \( x_{i3} \) are the coefficients of discrimination plane equation, \( c \) is a constant. Eq. (2) can be written as matrix form:

\[
Tx = c
\]

where \( T = [t_1; t_2; \ldots; t_n] \), \( x = [x_1; x_2; \ldots; x_n] \), superscript “\( \text{T} \)” stands for transport.

As we know, each LVs \( t_i \) is the linear combination of column vectors of \( X \) [27], that is


\[ T = XH \]  
where \( X \) is the data matrix and \( H \) is the weight matrix obtained by PLS decomposition.

- Inserting Eq. (4) into Eq. (3),

\[ XHx = c \]  
Let \( \beta = Hx \), so

\[ X\beta = c \]  
Then, the plane equation expressed by PLS LVs (scores) was transformed to that expressed by original variables. In the transformed equation (Eq. (6)), the absolute values of coefficients (\( \beta \)) can render the influence of corresponding variables on separation between sample classes. In turn, these compounds corresponding to these variables might be likely candidates for biomarkers.

3. Results and discussion

3.1. GC/MS profiles of plasma samples from DM-2 and health controls

Human plasma fatty acids were divided into two groups, EFA and NEFA. For most fatty acid analysis, only total plasma fatty acids or several fatty acids are taken into account in an experiment. Herein, we developed a two-step rapid method for comprehensive profiling of NEFA and EFA using KOH–CH\(_2\)OH to methylate EFAs followed by H\(_2\)SO\(_4\)–CH\(_3\)OH to methylate NEFAs. Then, GC/MS was applied to components isolation and detection. Method repeatability was evaluated by the analysis of five replicates of solid sample. The RSD of peak area ratios (analyte: I.S.) of 20 detected fatty acids in sample replicates were estimated to be 2.95–15.60%. Intra-day accuracy and precision (each, \( n = 5 \)) were evaluated as 4.17–5.09% RSD, by analysis of quality control (QC) samples (25, 250, 2500 \( \mu \)M of C18:1n-9 methyl ester) at different times during the same day. Inter-day accuracy and precision were determined as 7.77–8.84% RSD, by repeated analysis of QC samples over 5 consecutive days (\( n = 1 \) series per day). Furthermore, yields of the liquid–liquid extraction method and esterification process were assessed using C18:1n-9 and C18:1n-9 methyl ester standards and obtained the reaction yields of 95.22 ± 2.53% (\( n = 5 \)) and 96.96 ± 1.89% (\( n = 5 \)), respectively. Additional, internal standards, C17:0 and C17:0 methyl ester, were used to evaluate the extraction yields with matrix effect, which were 86.20 ± 3.87% (\( n = 3 \)) and 82.09 ± 1.25% (\( n = 3 \)). All results indicated that the method for the comprehensive profiling of NEFAs and EFAs was satisfactory.

Under the optimum analytical method, the metabolic profiles of EFA and NEFA from 78 DM-2 patients and 45 health controls were obtained by GC/MS, respectively. The NEFA profiles of DM-2 patients (Fig. 1A) and health controls (Fig. 1B) have shown that plasma free fatty acids of different people were almost the same, while the concentrations were different. Qualitative identification of these characteristic constituents was performed using retention times and mass spectra. Among the twenty detected fatty acids, six components, C16:1n-7, C18:1n-9, C18:1n-7, C18:2n-6, C18:3n-3 and C18:3n-6, were unequivocally determined by the corresponding standards according to their retention times and mass spectra. The other components were tentatively identified in chromatograms based on their electron bomb ionization (EI) MS data. Qualitative results were also shown in Fig. 1. Because the mass spectra of double-bond positional isomers and geometrical isomers are almost the same, some components which have isomers can not be identified in this experiment, such as C20:3, C22:5.

The obtained NEFA and EFA profiles have illustrated that the concentrations of fatty acids existed some differences between DM-2 patients and controls. However, because of the inter-human variation in plasma matrix composition and the complexity of the total ion chromatograms (TICs), coupled with differences in the content of fatty acids, visual comparison of those fatty acid profiles is very difficult. Hence, chemometric methods of multivariate statistical analysis were employed.

3.2. PCA of GC/MS data

First of all, each fraction of fatty acids was analyzed using PCA. Two scores plots of PC1 versus PC2, for NEFA and EFA profiles, showed separation between 78 DM-2 patients and 45 health controls. From Fig. 2, it is concluded that PCA analysis poorly distinguished between the DM-2 patients and health controls using NEFA profiles or EFA profiles. The two groups were scattered. However, when the variables were screened with the variable weight, much improvement was observed in the separation between two groups for NEFA profiles with 87.80% correct rate (Fig. 3). PCA, an unsupervised multivariate data analysis approach, is appropriate when it is believed that a function of many attributes is involved in differences between samples. However, the excessive irrelevant variables could result in confusion during the discrimination of samples [23,28]. For EFA profiles, the two groups were still scattered and could not be separated. The results indicated that EFAs might not reflect differences between DM-2 patients and controls, while the free fatty acids were suitable for classification. EFAs are from triglycerides, phospholipids, cholesterol esters and so on. This kind of fatty acids is relatively inactive. They are the stores of NEFA and will be released and turned into NEFAs. So, the alterations of NEFA should be the focus of our research. In the next PLS-LDA part, only NEFA profiles were taken into account.

3.3. The PLS-LDA model for discrimination of DM-2 and health controls

In the present study, we aimed to construct a model to visually discriminate type 2 diabetic patients and health controls and represent the variance of NEFA metabolic profiles of DM-2 patients in the therapeutic process with rosiglitazone. After overviewed the distribution of samples in the variable space by PCA, the supervised method, PLS-LDA was proposed to establish a visual model for the discrimination of DM-2 patients and controls. Here, linear discrimination analysis (LDA) was employed to construct the discriminate plane between two classes in the three-dimensional (3D) space. Variable scaling is an integral part of PLS-LDA as it regulates the relative importance of each variable in the subsequent model [29]. Autoscaling and centering are two widely used variable scaling methods [30,31]. Autoscaling not only centers the data set but also makes the standard variation of variable to be the same units. The separation and prediction ability of PLS-LDA model could be improved progressively from using just centering, after the application of autoscaling to the data set. The classification results are shown in Table 2. From Table 2 it can be seen that the
centered model showed relatively poor predictive ability with 76.67% prediction rate. The autoscaling model produced the higher prediction rate (83.33%). Additionally, the recognition rate (89.25%) and correct rate (87.80%) were both higher than those of the centered model (83.87% and 82.11%, respectively). In conclusion, the autoscaling method obviously improved the performance of PLS-LDA pattern recognition analysis and enhanced the predictive power of the model and was a good choice to optimize the class separation.

To construct a stable and credible model, 10 validation PLS-LDA models were calculated. Approximately three-fourths of the samples (the "training set") were selected at random to construct a PLS-LDA model that could be used to predict the class membership of the remaining one-fourth of samples (the "test set"). After comparison, the final 3D model of

Fig. 1. GC–MS profiles of free fatty acid methyl esters from plasma of the DM-2 patients (A) and health controls (B). (A) and (B) are the chromatograms of FAMES obtained by the optimum reaction condition and GC–MS condition. Those samples are derivatized and extracted from plasma of DM-2 patients and health controls, respectively. Furthermore, the qualitative results were marked in (A). Components in (A) could also be found in (B) sub-figures according to their retention times and mass spectra.
Fig. 2. PC scores plots derived from the total ion chromatograms (TIC) obtained from 78 DM-2 patients and 45 health controls before feature selection. Key: ● = health controls; ○ = DM-2 patients. (A) PC scores plots from TICs of NEFAs and (B) from TICs of EFAs. All data were autoscaled.

Fig. 3. PC scores plots of NEFAs generated from application of variable weight feature selection method to extract the informative variables. Key: ● = health controls; ○ = DM-2 patients.
Table 2
Classification of DM-2 patients and control samples by PLS-LDA\(^4\) method

<table>
<thead>
<tr>
<th>Method</th>
<th>Recognition rate</th>
<th>Prediction rate</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Correct rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centering</td>
<td>83.87%</td>
<td>76.67%</td>
<td>80.00%</td>
<td>83.33%</td>
<td>82.11%</td>
</tr>
<tr>
<td>78/93</td>
<td>23/30</td>
<td>36/45</td>
<td>65/78</td>
<td>101/123</td>
<td></td>
</tr>
<tr>
<td>Autoscaling</td>
<td>89.25%</td>
<td>83.33%</td>
<td>86.67%</td>
<td>88.46%</td>
<td>87.80%</td>
</tr>
<tr>
<td>83/93</td>
<td>25/30</td>
<td>39/45</td>
<td>69/78</td>
<td>108/123</td>
<td></td>
</tr>
</tbody>
</table>

\(^4\)Recognition rate is the correct classification of the training set. Prediction rate is the rate of the correct classification of the test set. Sensitivity is the number of true positives classified as positive. Specificity is the number of true negative classified as negative.

PLS-LDA was obtained which shown in Fig. 4. Samples of the test set (the hollow circles and diamonds in Fig. 4) were homogeneously distributed in the 3D space obtained by the first three LVs. The DM-2 and controls were separated clearly by the discriminate plane in both the training and test sets. The equation of the discrimination plane was:

\[
1.0t_1 + 1.167t_2 + 0.522t_3 + 1.108 = 0
\]

where \(t_1\), \(t_2\), and \(t_3\) were the first three LVs from PLS decomposition.

In conclusion, the efficient three dimensional visual model has been successfully constructed using feature selection, autoscaling and PLS-LDA.

3.4. Alterations of NEFA metabolic profiles of type 2 diabetic patients treated with rosiglitazone

Some publications have concluded that the total plasma NEFA levels would be significant lowered by rosiglitazone and other TZDs, none of them reported the metabolic free fatty acid profiles change track in long-term treatment [32,33]. In the present research, 10 obese type 2 diabetic patients, including six women and four men, were treated with rosiglitazone for about three months. Rosiglitazone is a hyperglycemic agent that works to lower the resistance to insulin in fat, liver and muscle cells and also by stopping abnormalities and dysfunctions in \(\beta\)-cells [24]. Herein, the constructed visual PLS-LDA model was employed to represent the variance of NEFA metabolic profiles in the therapeutic process. Thirty six NEFA metabolic profiles obtained by GC/MS were projected to the 3D space of PLS-LDA model. Fig. 5 represented the time-dependent alterations in the levels of free fatty acids following rosiglitazone treatment. Though the change tracks of every patient were not the same, there was a subtle but progressive trend to the discriminate plane between DM-2 and controls. There was an interesting phenomena demonstrated in Fig. 5. For most patients, such as s1, s3, s4, the plasma NEFA level increased after treated for two weeks. After that, the plasma NEFA level decreased obviously. It indicated that rosiglitazone does not lower plasma NEFA levels directly. The pathogenesis of DM-2 is very complicated. Further patients, their change tracks of free fatty acid metabolic profiles maybe have some differences, such as patient s6 and s7. The first two months, plasma NEFA levels of these two patients decreased just as other patients. While, at the third month, their plasma NEFA levels increased some. It inferred that the lipid metabolism of these two patients has some differences with others. This information will be very helpful for pathogenesis research of different people group in the future.

The 3D visual model showed us the plasma NEFA metabolic profiles change track of every patient clearly. The results further validated the efficiently of PLS-LDA model. It can not only discriminate the DM-2 and controls, but also represent the dynamic change track of NEFA profiles of patients after treated with medicine. This method provided a powerful tool for monitoring pharmacologic therapy, and will be a complement or an alternative to pathogenesis and pharmacodynamics research.

3.5. Potential biomarkers

The 3D PLS-LDA model was obtained by nine variables (C14:0, C16:0, C16:1n-9, C18:0, C18:1n-9, C18:1n-7, C20:3, C24:0, total NEFAs) screening with variance weight. Those nine variables have stronger influence than others for separation between DM-2 and controls. It is a crude screening of variables. In order to find out the key NEFAs which contribute to the discrimination of DM-2 and controls, say potential biomarkers, further screening among those nine variables have been taken.

In this study, the equation of the discrimination plane expressed by PLS LVs (Eq. (7)) was transformed to that expressed by original variables. In the transformed equation (Eq. (6)), the influence of original variables was represented by the absolute values of coefficients (\(\beta\) in Eq. (6)). So, the compounds corresponding to these variables might be likely candidates of biomarkers. Herein, absolute values of coefficients of the nine variables are shown in Fig. 6. The coefficients of C16:0, C18:0 and C18:1n-9 were obviously higher than other fatty acids. Those three components might be the poten-
tional biomarkers, which were firstly identified for discrimination DM-2 patients and health controls. Total NEFAs is the sum of the twenty detected NEFAs. In clinic, total NEFAs is an important index for diagnosis of DM-2. However, the absolute value of coefficient of total NEFAs was clearly lower than the three identified biomarkers. It inferred that using C16:0, C18:0 and C18:1n-9 as indexes may have more accurate result than total NEFAs in the diagnosis of DM-2.

Fig. 5. 3D-projection plots of 36 samples from 10 DM-2 patients collected at 0 (n = 10), 2 (n = 5), 4 (n = 7), 9 (n = 8) and 14 weeks (n = 6) after treated with rosiglitazone. The change tracks of ten DM-2 patients were mapped separately in (A)–(J). Those samples were projected to the 3D space obtained by PLS-LDA model. Key: ⊿ = DM-2 patients before treated (0 week); ● = 2 weeks; ○ = 4 weeks; ● = 9 weeks; □ = 14 weeks.
DM-2 is characterized by two major defects: a dysregulation of pancreatic hormone secretion and a decrease in insulin action on target tissues (insulin resistance). The defects in insulin action on target tissues are characterized by a decreased in muscle glucose uptake and by an increased hepatic glucose production. These abnormalities are related to several defects in insulin signaling mechanisms and in several steps regulating glucose metabolism (transport, key enzymes of glycogen synthesis or of mitochondrial oxidation) [34]. High circulating concentrations of NEFAs can amplify these postreceptors defects. Acute elevation of plasma NEFAs is necessary for insulin secretion. Chronic exposure to high levels of plasma NEFAs, however, leads to apoptosis of β-cells (lipotoxicity) [35,36] and is a major risk factor for cardiovascular disease and sudden death in patients with insulin resistance [19].

The three potential biomarkers, C16:0 (palmitic acid), C18:0 (stearic acid) and C18:1n-9 (oleic acid), are all very important bioactive molecules. They are not only the main energy source as nutrients, but also signaling molecules in various cellular processes. The long-term high level of these fatty acids in plasma may have more contribution to lipotoxicity than other NEFAs for their great influence on discrimination between DM-2 and controls. Some literatures have reported their relationship between insulin sensitivity, substrate oxidation, and so on [37–39]. The mechanism how NEFAs promote insulin secretion is not clearly understood, though one pathway has found out, that long-chain NEFAs amplify the insulin secretion from pancreatic β-cells by activating GPR40 [40–42]. Further researches should be done to find out their metabolic pathways and interaction with protein or other small molecules. It will be very helpful for pathogenesis research of DM-2 and finding new drug target. The three identified potential biomarkers may be the target components in the future mechanism research.

4. Conclusions

In this paper, a new strategy, metabolomics, was firstly applied to research of dynamic plasma fatty acid metabolic profiling and biomarkers of DM-2. The application of GC/MS coupled with PLS-LDA of data with variable weight scanning makes it possible to classify DM-2 patients and health controls and, further, to establish a 3D PLS-LDA model to visual represent alterations of NEFA metabolic profiles of type 2 diabetic patients treated with rosiglitazone for about 3 months. Furthermore, we discovered the potential biomarkers which could be identified by MS and corresponding standards.

The combination of multivariate approach and GC/MS data-mining metabolite identification program results in a very powerful tool for metabolomics research. This work has illustrated the potential of metabolomics to disease diagnose, pathogenesis and pharmacodynamics research.

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