

Original Article

Association of serum lipid metabolism with markers of urinary peptides in type 2 diabetes patients

Jia Li^{1*}, Guangzhen Fu^{2*}, Junjun Wang², Man Zhang^{1,2,3}

¹Clinical Laboratory Medicine, Beijing Shijitan Hospital, Capital Medical University, Beijing 100038, China; ²Clinical Laboratory Medicine, Beijing Shijitan Hospital, Peking University Ninth School of Clinical Medicine; Beijing Shijitan Hospital, Capital Medical University; Beijing Key Laboratory of Urinary Cellular Molecular Diagnostics, Beijing 100038, China; ³Chinese Medical Doctor Association of Lab Medicine, Beijing 100038, China. *Equal contributors and co-first authors.

Received October 14, 2015; Accepted November 28, 2015; Epub January 1, 2016; Published January 15, 2016

Abstract: Objective: To unveil the differently expressed urinary peptides that are associated with different stages of lipid control for type 2 diabetes mellitus. Methods: Patients with diagnosed type 2 diabetes mellitus (n=58) and normal controls (n=29) were included. Serum and urine samples were collected from these subjects. The fasting plasma glucose (FPG), hemoglobin A1c% (HbA1c), cholesterol (CHOL), triglyceride (TRIG), low-density lipoprotein (LDL), high-density lipoprotein (HDL) were measured by ADVIA2400 Chemistry System. These type 2 diabetic patients were divided into two groups according to CHOL, TRIG, HDL and LDL, respectively. Urinary peptides were condensed by magnetic beads based weak cation exchange chromatography and analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. ClinProt was used to profile and screen the polypeptide patterns based on different methods of grouping in diabetic patients and normal controls. Nano-liquid chromatography-tandem mass spectrometry was used to identify these urinary peptides. Results: Serum CHOL negatively correlated with urinary peptide m/z 2756.5 ($r=-0.315$, $P=0.016$). Serum LDL negatively correlated with urinary peptide m/z 2756.5 ($r=-0.390$, $P=0.002$). Areas under the receiver operating characteristic of the three peptides (m/z 2756.5, m/z 3223.2 and m/z 4753.8) were 0.731, 0.673 and 0.602, respectively. The above-mentioned peptide m/z 2756.5 was further identified as fragment of fibrinogen alpha chain precursor and m/z 3223.2 was fragment of prothrombin precursor. Conclusion: There were three urinary peptides (m/z 2756.5, m/z 3223.2 and m/z 4753.8) that differently expressed in different stages of serum lipid control in type 2 diabetes mellitus.

Keywords: Urinary peptides, type 2 diabetes mellitus, dyslipidaemia, MALDI-TOF MS

Introduction

In recent decades, there has been an unprecedented rise in the prevalence of diabetes. The International Diabetes Federation foresees an increase of epidemic proportions in type 2 diabetes from about 387 million today to 592 million by 2035 [1]. The number of adults with diabetes is expected to increase by 69% in developing countries from 2010 to 2030 [2]. These escalating rates of diabetes worldwide represent a heavy disease burden at the population and individual level as well as for the total health care system.

Insulin resistance is a main characteristic of type 2 diabetes mellitus. The directly result of insulin resistance is the dysfunctional plasma

glucose metabolism. However, insulin resistance can also lead to alterations in the regulation of plasma lipid metabolism which is a major contributor to the macro-vascular disease associated with type 2 diabetes mellitus [3-5]. Vascular disease is the major cause of morbidity and mortality in patients with type 2 diabetes mellitus [6, 7]. So, it's imperative to have a better lipid metabolism control for diabetes patients though the use of a more convenient, reliable test. However, there still is not enough evidence to systematically expound pathobiological mechanism for diabetes under the current state of research.

Urine has long been a promising body fluid for study, not only in biomarker research but also in clinical diagnosis. In comparison to plasma,

urine has many advantages. First, urine can be collected noninvasively, frequently, and in large quantities. Second, protein or peptides in urine is more stable than that in plasma for proteases are activated during blood collection while the proteolytic degradation process is finished before urine collection [8, 9]. For example, there is no significant changes of urinary proteins after urine samples are stored for three days at 4°C or for 6 h at room temperature [10]. Third, we can collect urine samples over a period of time. Thus, it is convenient to monitor time-dependent changes. Most importantly, urine is the result of being a filtrate of blood and collecting all wastes from the body. There are researches indicate that small molecules are abundant in urine [11] and thousands of different kinds of proteins are present in urine [12, 13]. Therefore, urine reflects not only kidney disease but also systemic physiology.

The changes of urinary protein and peptides could reflect the metabolic state of the body. Our study had found two urinary peptides that associated closely with the level of glycated hemoglobin in type 2 diabetes mellitus [14]. Dyslipidaemia is common in patients with type 2 diabetes mellitus. So, the aim of this study was to unveil the differently expressed urinary peptides that are associated with different stages of lipid control for type 2 diabetes mellitus, which, to our knowledge, has not yet been explored.

Materials and methods

Study subjects

Study participants included 29 normal controls and 58 patients with diagnosed type 2 diabetes mellitus. Participants were recruited from Beijing Shijitan Hospital from February 2013 until June 2013. The participants all gave written informed consent, which was in accordance with the provisions of the Helsinki Declaration and approved by the ethics committee of Beijing Shijitan Hospital, Capital Medical University. Individuals with type 2 diabetes, defined as the use of oral glucose-lowering treatment or a fasting plasma glucose >7.0 mmol/l (126 mg/dl) or nonfasting plasma glucose >11.1 mmol/l (>200 mg/dl), were eligible for this study. Exclusion criteria included any diagnoses of renal disease, or acute or chronic inflammatory disease. Normal controls were

chosen after a clinical check of renal function, blood pressure, microalbuminuria and urinary sediment.

These 58 type 2 diabetic patients were divided into two groups according to the serum level of CHOL, TRIG, HDL and LDL, respectively. Firstly, grouping type 2 diabetes mellitus patients based on the serum level of CHOL. Type 2 diabetic patient with CHOL less than 5.3 mmol/L was defined as CM1 and that greater than 5.3 mmol/L was defined as CM2. Then, grouping type 2 diabetes mellitus patients based on the serum level of TRIG. Type 2 diabetic patients with TRIG less than 1.7 mmol/L was defined as TM1 and that greater than 1.7 mmol/L was defined as TM2. Thirdly, grouping type 2 diabetes mellitus patients based on the serum level of HDL. Type 2 diabetic patient with HDL less than 1.2 mmol/L was defined as HM1 and that greater than 1.2 mmol/L was defined as HM2. Lastly, grouping type 2 diabetes mellitus patients based on the serum level of LDL. Type 2 diabetic patient with LDL less than 3.0 mmol/L was defined as LM1 and that greater than 3.0 mmol/L was defined as LM2. N represents normal controls.

The clinical characteristics of normal controls and type 2 diabetes patients are shown in **Table 1**.

Serum and urine samples collection and preparation

After a 12-h fasting period, venous blood specimens were collected in plain tubes, centrifuged to yield serum and stored at -80°C until analysis.

Random midstream urine samples were collected in the morning into sterile polypropylene tubes. Immediately after collection, urine samples were centrifuged at 400×g for 5 min to remove cell debris and casts. Then supernatants were divided in aliquots and frozen at -80°C refrigeration until use.

Serum parameters profile

Fasting plasma glucose (FPG), hemoglobin A1c% (HbA1c), fasting serum total cholesterol (CHOL), triglyceride (TRIG), low-density lipoprotein (LDL), high-density lipoprotein (HDL) were measured by ADVIA2400 Chemistry System

Urinary peptides in type 2 diabetes mellitus

Table 1. Clinical data of diabetic patients and normal controls

Group	Number (n)	Gender (M/F)	Age (year)	FPG (mmol/L)	HbA1c% (mmol/mol)	Duration (year)
N	29	23/6	50.93±9.94	5.49±0.35	5.2±0.3 (33±4)	/
DM	58	37/21	59.79±12.07	9.04±2.03	7.6±1.2 (60±13)	6.03±6.19
DM grouped by CHOL						
CM1	31	20/11	64.03±10.63	8.78±2.01	7.5±1.1 (58±13)	7.30±6.58
CM2	27	17/10	54.92±11.96	9.34±2.05	7.8±1.3 (61±14)	4.58±5.47
DM grouped by TRIG						
TM1	41	27/14	60.76±11.39	8.98±1.87	7.6±1.3 (60±14)	6.29±6.31
TM2	17	10/7	57.47±13.66	9.19±2.42	7.6±1.2 (59±13)	5.41±6.03
DM grouped by HDL						
HM1	21	17/4	61.24±11.11	9.53±2.30	8.1±1.4 (64±15)	6.49±5.20
HM2	37	20/17	58.97±12.66	8.77±1.84	7.3±1.0 (56±11)	5.78±6.75
DM grouped by LDL						
LM1	29	18/11	64.27±11.06	8.84±2.08	7.3±1.0 (57±11)	7.36±6.87
LM2	29	19/10	55.31±11.52	9.25±1.99	7.9±1.4 (62±15)	4.71±5.21

(Siemens Healthcare Diagnostics, Deerfield, IL, USA).

Urinary peptides profile

Prior to urine peptides isolation, urine samples were thawed at 4°C followed by centrifugation at 3000 rpm for 10 min, and then supernatants were immediately proceeded to peptides fractionation.

We used weak cationic-exchange magnetic beads (MB-WCX) to separate and purify urinary peptides through binding, washing, and elution according to the manufacturer's instructions (Bruker Daltonics). First, 10 µl MB-WCX and 95 µl MB-WCX binding solution were added in a polypropylene tube, mixed thoroughly and then 10 µl urine samples were added and mixed thoroughly. The tubes were placed in a magnetic bead separator (Bruker Daltonics) to separate the unbound solution. The magnetic beads were then washed three times. Second, 10 µl MB-WCX eluting solution was added and mixed intensively by vortexing. Finally, the clear supernatant was transferred into a fresh tube, and 5 µl MB-WCX stabilizing solution was added. The well-mixed eluate was then stored at -20°C.

The eluate of urinary sample was diluted 1:10 in matrix solution containing α-cyano-4-hydroxycinnamic acid (Bruker Daltonics). Then 1 µl of the resulting mixture was spotted on-

to the AnchorChip target (Bruker Daltonics), allowed to air dry and ionized by a nitrogen laser ($\lambda = 337$ nm) operating at 25 Hz. MALDI-TOF MS was performed using an Autoflex TOF instrument (Bruker Daltonics). Before data acquisition of every eight samples, the standard preparation would be calibrated. Eleven peptides were used as external standard preparation. Then, mass calibration was performed. For each MALDI spot, 400 spectra were acquired in analysis (50 laser shot at 8 different spot positions) and the average of 8 spots represents one urine sample.

Data processing and statistical analysis

The spectra of all signals with a signal-to-noise ratio >5 were collected in the mass range of 1000-10000Da. All the spectra were analyzed using ClinProTools2.1 software to normalize spectra (using total ion count), subtract baseline and determine peak m/z values and intensities. To align the spectra, a mass shift of no more than 0.1% was determined. The peak area was used as quantitative standardization.

ClinProTools2.1 bioinformatics software was used to find out the differently expressed urinary peptides. To avoid deviation, only peak with average area >1000 in either of three groups were analyzed in our study. Comparative analysis was performed four times, one among CM1, CM2 and N, one among TM1, TM2 and N,

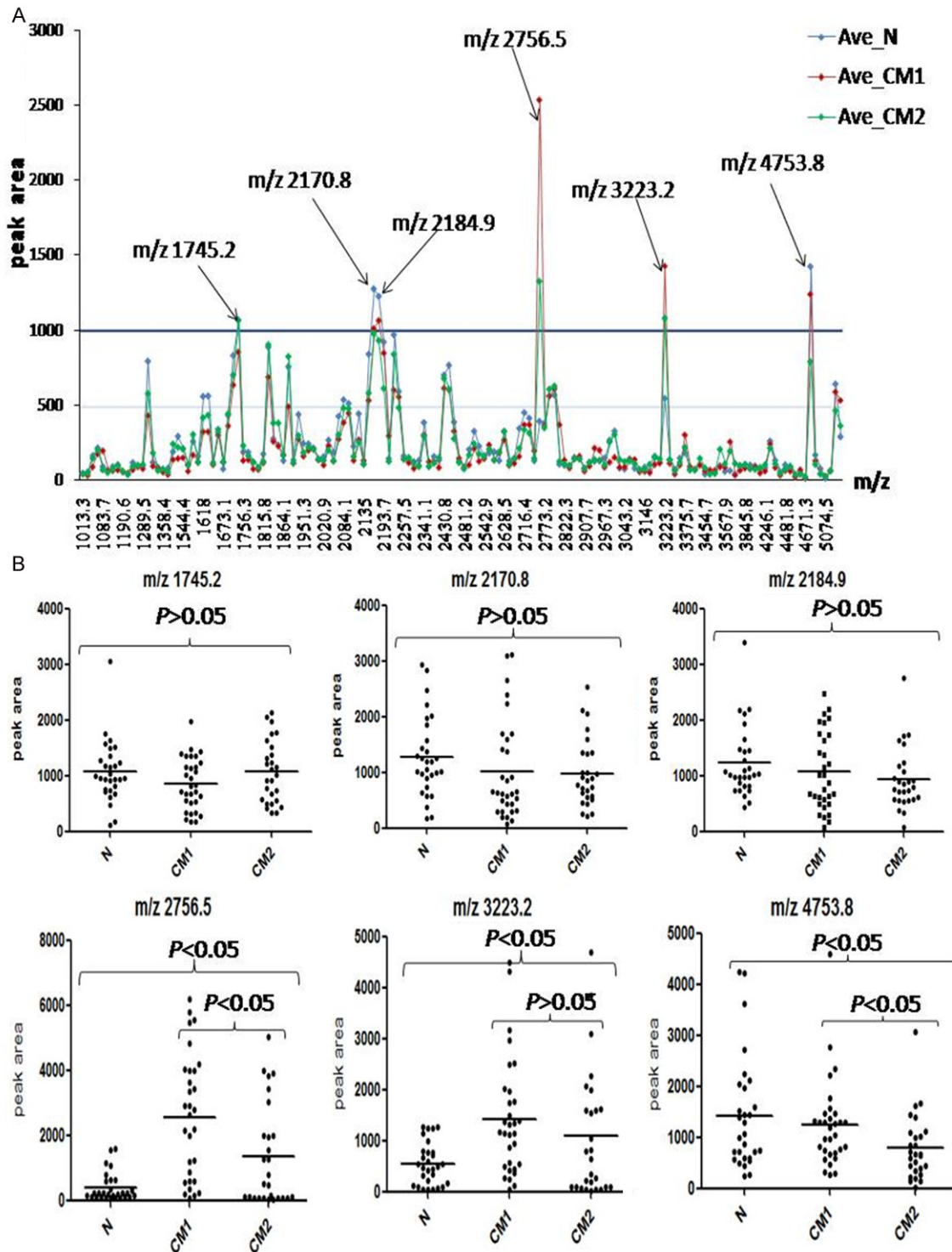


Figure 1. Urine peptidome and data analysis of type 2 diabetes patients and normal controls based on CHOL. A. The average intensity of 152 peaks that were detected by ClinprotTools2.1 available in the 1,000 to 10,000 m/z range. Arrows indicate peaks with average area >1000 in either of three groups. B. The distributions of the six peaks in all samples of the three groups. The intensities of peak m/z 2756.5, m/z 3223.2 and m/z 4753.8 were statistically significant among three groups ($P < 0.05$). Peaks area of m/z 2756.5 and m/z 4753.8 was statistically significant between CM1 and CM2 ($P < 0.05$).

one among HM1, HM2 and N, and another among LM1, LM2 and N. The comparison of the m/z values among three groups was performed by nonparametric tests: the Mann-Whitney U test (for binary comparisons) and the Kruskal-Wallis test (for multi-group comparisons) using ClinProTools2.1 bioinformatics software. In all cases two-tailed $P < 0.05$ was accepted as statistically significant.

Receiver operating characteristic (ROC) curve analysis and area under the curve (AUC) calculations were performed directly with SPSS17.0 software to determine diagnostic efficacy of each single marker. Correlation analysis was performed to examine the relationship between the major urinary peptides and the level of serum lipid metabolism with SPSS17.0 software.

Identification of urinary peptide

Nano-liquid chromatography-tandem mass spectrometry, which consisted of an Aquity UPLC system (Waters) and a LTQ Orbitrap XL mass spectrometer (Thermo Fisher) equipped with a nano-ESI source, was used to identify the sequences of differential expression peptides. Firstly the peptide solutions were loaded to a C18 trap column (symmetry 180 $\mu\text{m} \times 20 \text{ mm} \times 5 \mu\text{m}$, nano Acquity) with the flow rate of 15 $\mu\text{l}/\text{min}$ for 3 min. Then the desalted peptides were analyzed by C18 analytical column (symmetry 75 $\mu\text{m} \times 150 \mu\text{m} \times 3.5 \mu\text{m}$, nano Acquity) at a flow rate of 400 nL/min. The mobile phases A (5% acetonitrile, 0.1% formic acid, Sigma-Aldrich) and B (95% acetonitrile, 0.1% formic acid) were used for analytical columns. Gradient elution profile was as follows: 5% B from initial to 40 min, 45% B from 40 min to 41 min, 80% B from 41 min to 45 min, 80% B from 45 min to 45.5 min, 5% B from 45.5 min to 60 min, and 5% B in 60 min. The MS instrument was operated in a data-dependent model. The range of full scan was 400-2000 m/z with a mass resolution of 100,000 (m/z 400). The ten most intense monoisotope ions were the precursors for collision induced dissociation for two consecutive scans per precursor ion followed by 90 s of dynamic exclusion.

The obtained spectra were analyzed with BioworksBrowser3.3.1 SP1 (Thermo Fisher) and the information was matched against the

IPI Human database (v3.45) using Sequest search. Parameters were set as follows: Delton ≥ 0.1 ; Charge2+, Xcorr2.0; charge3+, Xcorr2.5; peptide probability $\leq 1 \times 10^{-3}$; parent ion masses tolerance: 50 ppm; fragment ion masses tolerance: 1 Da; enzyme: no enzyme; variable modification: oxidation of methionine.

Results

Urine peptidome and data analysis of type 2 diabetes patients and normal controls based on CHOL

Following the grouping as described in methods, the average intensity of 152 peaks that were detected by ClinProTools2.1 available in the 1,000 to 10,000 m/z range is shown in **Figure 1A**. Arrows indicate peaks with average area > 1000 in either of three groups and their distributions in all samples of the three groups are shown in **Figure 1B**. The statistical analysis of these peaks is also shown in **Figure 1B**. The intensity of peak m/z 2756.5, m/z 3223.2 and m/z 4753.8 was statistically significant among three groups ($P < 0.05$). Peaks area of m/z 2756.5 and m/z 4753.8 was statistically significant between CM1 and CM2 ($P < 0.05$).

Urine peptidome and data analysis of type 2 diabetes patients and normal controls based on TRIG

Following the grouping as described in methods, the average intensity of 152 peaks that were detected by ClinProTools2.1 available in the 1,000 to 10,000 m/z range is shown in **Figure 2A**. Arrows indicate peaks with average area > 1000 in either of three groups and their distributions in all samples of the three groups are shown in **Figure 2B**. The statistical analysis of these peaks is also shown in **Figure 2B**. The intensity of peak m/z 2756.5, m/z 3223.2 and m/z 4753.8 was statistically significant among three groups ($P < 0.05$).

Urine peptidome and data analysis of type 2 diabetes patients and normal controls based on HDL

Following the grouping as described in methods, the average intensity of 152 peaks that were detected by ClinProTools2.1 available in the 1,000 to 10,000 m/z range is shown in

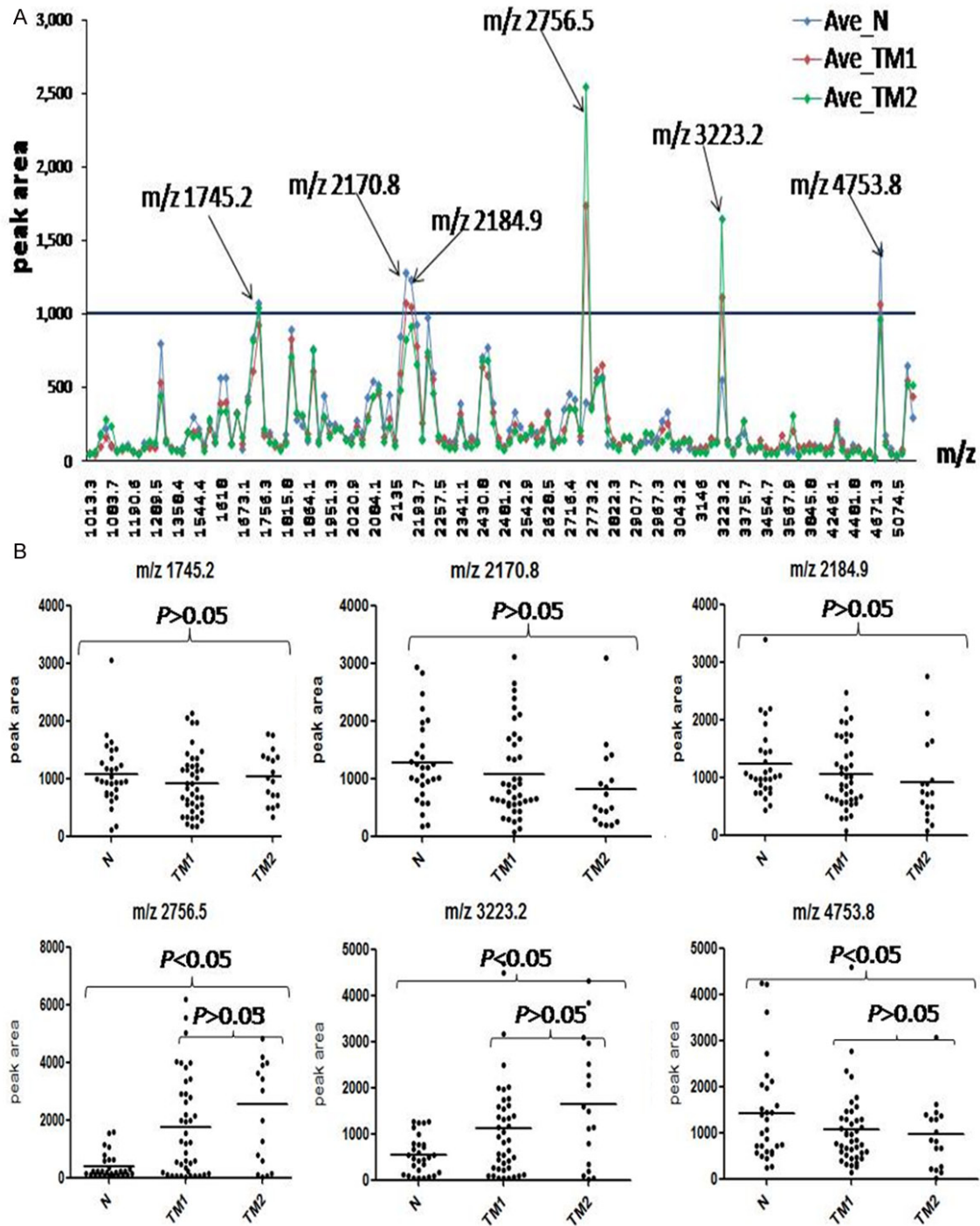


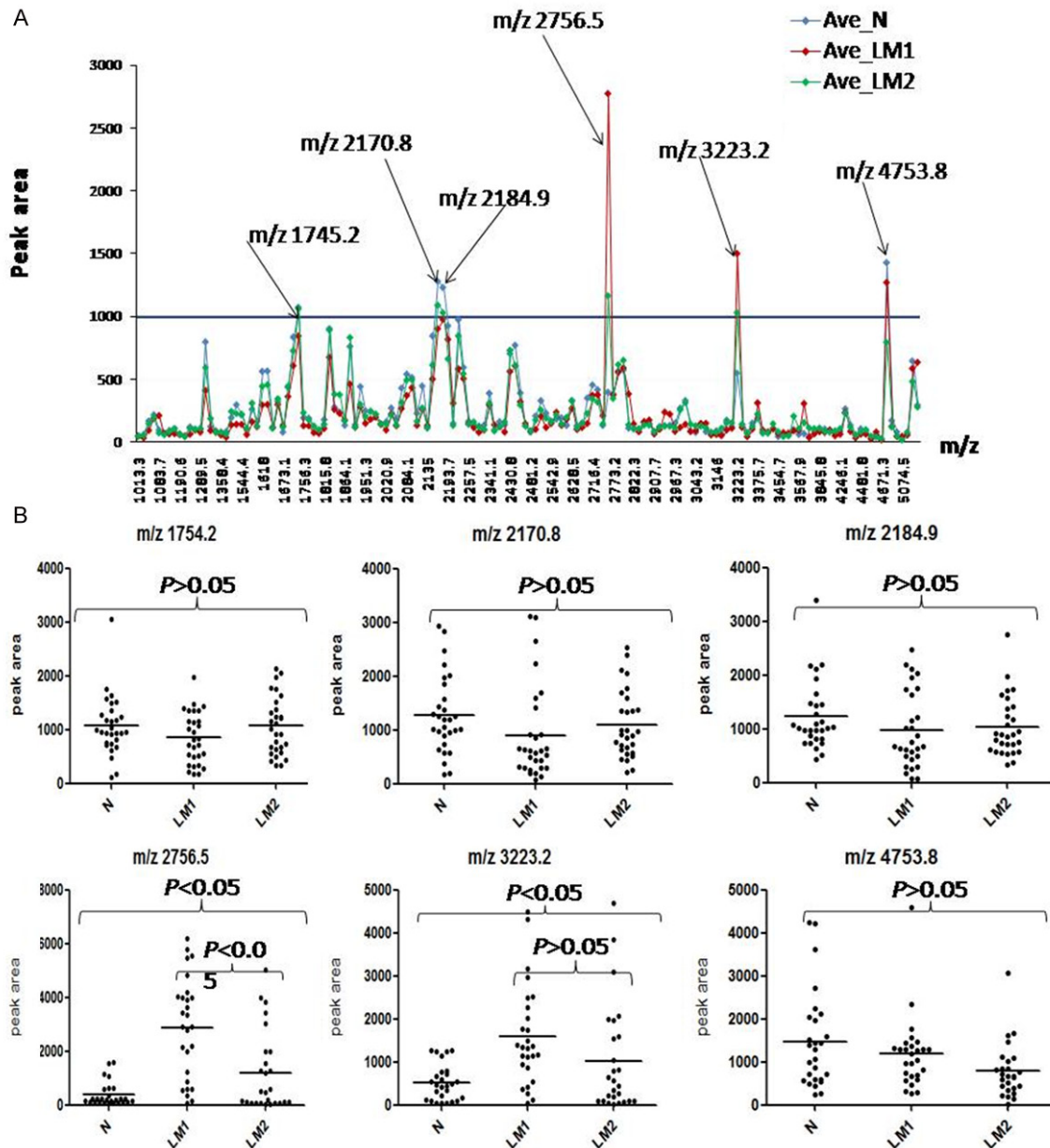
Figure 2. Urine peptidome and data analysis of type 2 diabetes patients and normal controls based on TRIG. A. The average intensity of 152 peaks that were detected by ClinprotTools2.1 available in the 1,000 to 10,000 m/z range. Arrows indicate peaks with average area >1000 in either of three groups. B. The distributions of the six peaks in all samples of the three groups. The intensities of peak m/z 2756.5, m/z 3223.2 and m/z 4753.8 were statistically significant among three groups ($P < 0.05$).

Figure 3A. Arrows indicate peaks with average area >1000 in either of three groups and their distributions in all samples of the three groups

are shown in **Figure 3B**. The statistical analysis of these peaks is also shown in **Figure 3B**. The intensity of peak m/z 2756.5 and m/z 3223.2

was statistically significant among three groups ($P < 0.05$). Peaks area of m/z 3223.2 was statis-

Int J Clin Exp Pathol 2016;9(1):37-48



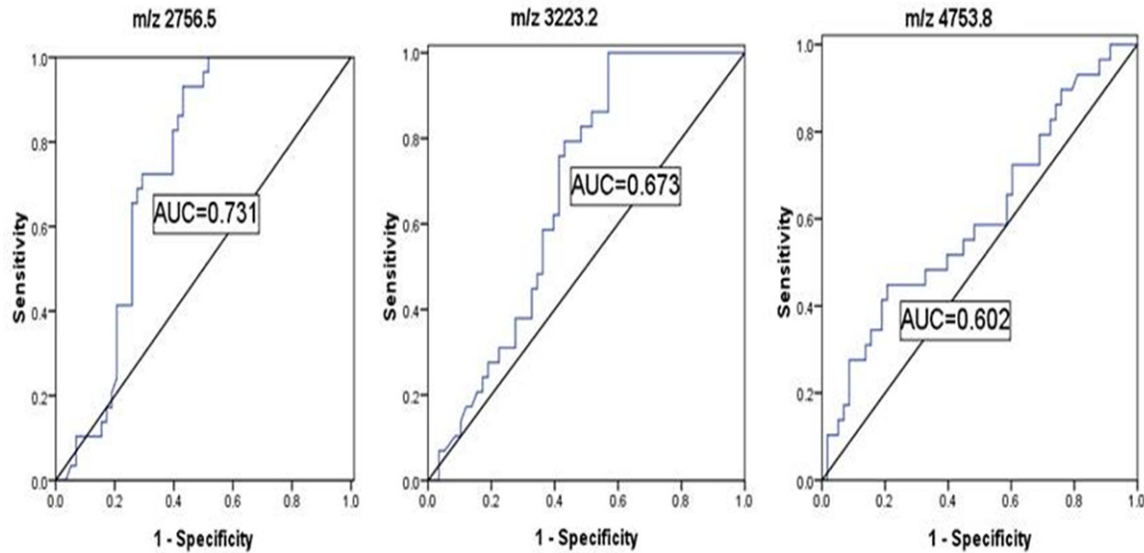


Figure 5. ROC curve and area under the curve (AUC) of the differently expressed peaks.

Table 2. Identified peptides sequences of the differently expressed peaks

m/z	Molecular weight	Amino sequences	Protein name and corresponding IPI
2756.5	2756.22	S.SYSKQFTSSTSYNRGDSTFESKSY.K	IPI00021885 Isoform 1 of Fibrinogen alpha chain precursor
3223.2	3220.74	C.GLRPLFEKKSLEDKTERELLESYIDGR.I	IPI00019568 Prothrombin precursor
4753.8		Identification failure	

2756.5 was statistically significant between HM1 and HM2 ($P < 0.05$).

ROC analysis

Receiver operating characteristic (ROC) curve and area under the curve (AUC) of the three peaks are shown in **Figure 5**. AUC of m/z 2756.5, m/z 3223.2, and m/z 4753.8 was 0.731, 0.673 and 0.602, respectively.

Relationship of these three urinary peptides with serum lipid metabolism

Correlation analysis was performed to examine the relationship between the major urinary peptides and serum lipid metabolism. Serum CHOL negatively correlated with m/z 2756.5 ($r = -0.315$, $P = 0.016$), m/z 3223.2 ($r = -0.139$, $P = 0.297$) and m/z 4753.8 ($r = -0.199$, $P = 0.135$). Serum TRIG correlated positively with urinary peak m/z 2756.5 ($r = 0.093$, $P = 0.487$) and m/z 3223.2 ($r = 0.210$, $P = 0.114$) and negatively with m/z 4753.8 ($r = -0.221$, $P = 0.095$). Serum HDL positively correlated with urinary peak m/z 4753.8 ($r = 0.122$, $P = 0.362$) and negatively with

m/z 2756.5 ($r = -0.065$, $P = 0.627$) and m/z 3223.2 ($r = -0.185$, $P = 0.165$). Serum LDL negatively correlated with m/z 2756.5 ($r = -0.390$, $P = 0.002$), m/z 3223.2 ($r = -0.199$, $P = 0.135$) and m/z 4753.8 ($r = -0.249$, $P = 0.06$).

Identification of the four peptides

By nano-liquid chromatography-tandem mass spectrometry detection, the peptide sequences of the differential peaks were identified. The amino acid sequences of m/z 2756.1 SYS-KQFTSSTSYNRGDSTFESKSY and m/z 3223.2 GLRPLFEKKSLEDKTERELLESYIDGR. Unfortunately, m/z 4753.8 was not identified. Through the Sequest search, m/z 2756.5 was fragment of fibrinogen alpha chain precursor and m/z 3223.2 was found to be partial sequences of prothrombin precursor. The complete identification results are shown in **Table 2**.

Discussion

Lipid abnormalities are common in people with type 2 diabetes mellitus. Recent developments

have recognized the complex nature of diabetic dyslipidaemia is characterized by a spectrum of quantitative and qualitative changes in lipid and lipoproteins which encompasses elevated plasma concentrations of triglyceride (TRIG), small dense low-density lipoprotein (LDL) and low high-density lipoprotein (HDL) [15]. However, the prevalence of dyslipidemia in type 2 diabetes varies in different study populations based on the presence of metabolic syndrome and the variable definition of the cut off levels for serum lipid [16, 17]. The FIELD study reported about 38% of the recruited subjects had dyslipidemia (triglycerides >1.7 mmol/L and low HDL<1.03 mmol/L in men and <1.29 mmol/L in women [18]. There was a larger population based registry of 75048 patients with type 2 diabetes in Sweden reported that about 37-38% had elevated triglycerides (>1.7 mmol/L but <4.0 mmol/L) with or without low HDL [19]. In our study, it was about 30% of the subjects that had elevated triglycerides (>1.7 mmol/L) with or without low HDL.

A number of factors may contribute to lipid abnormalities in patients with type 2 diabetes, including insulin resistance, adipocytokines, and hyperglucaemia [20-22]. There was study indicating that triglycerides and unesterified cholesterol are markers of poor glycemic control in patients with type 2 diabetes [23]. However, elevated plasma TRIG, small dense LDL and low HDL can be detected years before the clinical diagnosis of type 2 diabetes in subjects with normal glucose concentrations [24]. Hyperglucaemia can lead to dyslipidaemia while dyslipidaemia may also present before its diagnosis in patients with type 2 diabetes. The underlying pathophysiology of diabetic dyslipidemia is complex and still not well understood.

With the development of mass spectra technology, urinary proteomics has emerged as a promising tool for biomarker screening and identification. Bead based MALDI-TOF MS allows to enrich and analysis of the small proteins or peptides in different bio fluids, so the lower molecular mass compounds in the urine can now be analyzed in a mass spectrometer without additional manipulation (e.g. tryptic digests) [25]. Therefore, analysis of a urinary lower molecular mass peptide would be more prone to regularly and systematically diagnosis of disease and reveal pathophysiology of the disease.

To avoid deviation, only peak with average area >1000 in any one of the three groups were analyzed in our study. Diabetic dyslipidaemia consists of elevated plasma concentrations of TRIG, small dense LDL and low HDL. So, comparative analysis was performed four times in our study. First, patients with type 2 diabetes were divided into two groups based on the serum level of CHOL. **Figure 1A** shows that there were six peaks of which average area >1000 in any one of the three groups. **Figure 1B** shows the intensity of peaks m/z 2756.5, m/z 3223.2 and m/z 4753.8 had statistical difference among three groups, but there was no significant difference between CM1 and CM2 for peak of m/z 3223.2. Second, we divided diabetes patients into two groups based on the serum level of TRIG. **Figure 2A** shows that there were six peaks of which average area >1000 in any one of the three groups. **Figure 2B** shows the intensity of peaks m/z 2756.5, m/z 3223.2 and m/z 4753.8 had statistical difference among three groups, but the intensity of the three peaks had no significance difference between TM1 and TM2. Third, we divided diabetes patients into two groups based on the serum level of HDL. **Figure 3A** shows that there were six peaks of which average area >1000 in any one of the three groups. **Figure 3B** shows the intensity of peaks m/z 2756.5 and m/z 3223.2 had statistical difference among three groups, but the intensity of the peak m/z 2756.5 had no significance difference between HM1 and HM2. Fourth, we divided diabetes patients into two groups based on the serum level of LDL. **Figure 4A** shows that there were six peaks of which average area >1000 in any one of the three groups. **Figure 4B** shows the intensity of peaks m/z 2756.5 and m/z 3223.2 had statistical difference among three groups, but the intensity of the peak m/z 3223.2 had no significance difference between LM1 and LM2.

There were six peaks with average area >1000 in any one of the three groups based on different grouping methods. However, they present different statistical characters. When correlation analysis was performed to examine the relationship between the major urinary peptides and serum lipid metabolism, only the intensity of peak m/z 2756.5 was negatively correlated with the serum level of CHOL and LDL.

The peak of m/z 2756.5 was identified as a fragment of isoform 1 of fibrinogen alpha chain precursor and m/z 2756.5 was a fragment of prothrombin precursor. We know that fibrinogen and prothrombin are coagulation factors in blood. But, it is unclear whether these proteins play roles in other process of pathophysiology in type 2 diabetes and we didn't know how these fragments filtrate into urine. Urine is partial ultrafiltrate of blood and it is a window to reflect the actual changes of the body.

In type 2 diabetes mellitus, there are some major pathological conditions that could be responsible for the presence of diabetes-related biomarkers in urine: oxidative stress, low-grade inflammation and endothelial damage [26]. There exist lipid and protein metabolic disorders in type 2 diabetes mellitus. Increased lipid synthesis further stimulates artery smooth muscle cell proliferation, leading to vascular basement membrane thickening and resulting in microcirculation disturbance. It is easy to cause tissue ischemia, hypoxia and produce a large number of reactive oxygen species (ROS) [27-29]. ROS could injure vascular intimal membrane and put the patients with type 2 diabetes in a pathological state of blood coagulation and fibrinolysis. Under this state, the signals could induce the corresponding cell to produce more proteins [30]. The two elevated urinary peptides represent main clinical parameters for the evaluation of the diabetic status which may be used as indicators of risk for diabetic vascular complications.

Acknowledgements

We gratefully acknowledge all volunteers for generous donation of urine samples as well as the staffs in the clinical laboratory of Beijing Shijitan Hospital for their enthusiastic support. Our study was funded by National High Technology Research and Development Program of China (2014AA020901) and Beijing Key Laboratory of Urinary Cellular Molecular Diagnostics (Z151100001615060).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Man Zhang, Clinical Laboratory Medicine, Beijing Shijitan Hospital, Capital Medical University, 10 Tieyi Road, Haidian District, Beijing 100038, China. Tel: 0086 10 6392-

6389; Fax: 0086 10 63926283; E-mail: mzhang99@aliyun.com

References

- [1] http://www.idf.org/sites/default/files/Atlas-poster-2014_EN.pdf.
- [2] Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010; 87: 4-14.
- [3] Turner RC, Millns H, Neil HA, Stratton IM, Manley SE, Matthews DR, Holman RR. Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23). *BMJ* 1998; 316: 823-828.
- [4] McEwen LN, Karter AJ, Waitzfelder BE, Marrero DG, Mangione CM, Herman WH. Predictors of mortality over 8 years in type 2 diabetic patients: Translating Research into Action for Diabetes (TRIAD). *Diabetes Care* 2012; 35: 1301-1309.
- [5] Eliasson B, Cederholm J, Eeg-Olofsson K, Svensson AM, Zethelius B, Gudbjornsdottir S. Clinical usefulness of different lipid measures for prediction of coronary heart disease in type 2 diabetes: a report from the Swedish National Diabetes Register. *Diabetes Care* 2011; 34: 2095-2100.
- [6] Norgaard ML, Andersen SS, Schramm TK, Jørgensen CH, Hansen ML, Andersson C, Bretler DM, Vaag A, Køber L, Torp-Pedersen C, Gislason GH. Changes in short- and long-term cardiovascular risk of incident diabetes and incident myocardial infarction—a nationwide study. *Diabetologia* 2010; 53: 1612-1619.
- [7] Mulnier HE, Seaman HE, Raleigh VS, Colhoun HM, Lawrenson RA, de Vries CS. Risk of myocardial infarction in men and women with type 2 diabetes in the UK: a cohort study using the General Practice Research Database. *Diabetologia* 2008; 51: 1639-1645.
- [8] Decramer S, Gonzalez de Peredo A, Breuil B, Monsarrat B, Bascands JL, Schanstra JP. Urine in clinical proteomics. *Mol Cell Proteomics* 2008; 7: 1850-1862.
- [9] Yi J, Kim C, Gelfand CA. Inhibition of intrinsic proteolytic activities moderates preanalytical variability and instability of human plasma. *J Proteome Res* 2007; 6: 1768-1781.
- [10] Schaub S, Wilkins J, Weiler T, Sangster K, Rush D, Nickerson P. Urine protein profiling with surface-enhanced laser desorption/ionization time-of-flight mass spectrometry. *Kidney Int* 2004; 65: 323-332.
- [11] Bouatra S, Aziat F, Mandal R, Guo AC, Wilson MR, Knox C, Bjorn Dahl TC, Krishnamurthy R, Saleem F, Liu P, Dame ZT, Poelzer J, Huynh J,

- Yallou FS, Psychogios N, Dong E, Bogumil R, Roehring C, Wishart DS. The human urine metabolome. *PLoS One* 2013; 8: e73076.
- [12] Marimuthu A, O'Meally RN, Chaerkady R, Subbannayya Y, Nanjappa V, Kumar P, Kelkar DS, Pinto SM, Sharma R, Renuse S, Goel R, Christopher R, Delanghe B, Cole RN, Harsha HC, Pandey A. A comprehensive map of the human urinary proteome. *J Proteome Res* 2011; 10: 2734-2743.
- [13] Kentsis A, Monigatti F, Dorff K, Campagne F, Bachur R, Steen H. Urine proteomics for profiling of human disease using high accuracy mass spectrometry. *Proteomics Clin Appl* 2009; 3: 1052-1061.
- [14] Zhang M, Fu G, Lei T. Two urinary peptides associated closely with type 2 diabetes mellitus. *PloS One* 2015; 10: e0122950.
- [15] Arca M, Pigna G, Favoccia C. Mechanisms of diabetic dyslipidemia: relevance for atherogenesis. *Curr Vasc Pharmacol* 2012; 10: 684-686.
- [16] Taskinen MR. Type 2 diabetes as a lipid disorder. *Curr Mol Med* 2005; 5: 297-308.
- [17] Chapman MJ, Ginsberg HN, Amarenco P, Andreotti F, Borén J, Catapano AL, Descamps OS, Fisher E, Kovanen PT, Kuivenhoven JA, Lesnik P, Masana L, Nordestgaard BG, Ray KK, Reiner Z, Taskinen MR, Tokgözoğlu L, Tybjaerg-Hansen A, Watts GF; European Atherosclerosis Society Consensus Panel. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. *Eur Heart J* 2011; 32: 1345-1361.
- [18] Scott R, Donoghoe M, Watts GF, O'Brien R, Pardy C, Taskinen MR, Davis TM, Colman PG, Manning P, Fulcher G, Keech AC; FIELD Study Investigators. Impact of metabolic syndrome and its components on cardiovascular disease event rates in 4900 patients with type 2 diabetes assigned to placebo in the FIELD randomised trial. *Cardiovasc. Diabetol* 2011; 10: 102.
- [19] Eriksson M, Zethelius B, Eeg-Olofsson K, Nilsson PM, Gudbjörnsdóttir S, Cederholm J, Eliasson B. Blood lipids in 75,048 type 2 diabetic patients: a population-based survey from the Swedish National Diabetes Register. *Eur J Cardiovasc Prev Rehabil* 2011; 18: 97-105.
- [20] Taskinen MR. Diabetic dyslipidaemia: from basic research to clinical practice. *Diabetologia* 2003; 46: 733-749.
- [21] Chahil TJ, Ginsberg HN. Diabetic dyslipidemia. *Endocrinol Metab Clin North Am* 2006; 35: 491-510, vii-viii.
- [22] Vergès B. New insight into the pathophysiology of lipid abnormalities in type 2 diabetes. *Diabetes Metab* 2005; 31: 429-439.
- [23] Laverdy OG, Hueb WA, Sprandel MC, Kalil-Filho R, Maranhão RC. Effects of Glycemic Control upon Serum Lipids and Lipid Transfers to HDL in Patients with Type 2 Diabetes Mellitus: Novel Findings in Unesterified Cholesterol Status. *Exp Clin Endocrinol Diabetes* 2015; 123: 232-239.
- [24] Lorenzo C, Hartnett S, Hanley AJ, Rewers MJ, Wagenknecht LE, Karter AJ, Haffner SM. Impaired fasting glucose and impaired glucose tolerance have distinct lipoprotein and apolipoprotein changes: the insulin resistance atherosclerosis study. *J Clin Endocrinol Metab* 2013; 98: 1622-1630.
- [25] Decramer S, Gonzalez de Peredo A, Breuil B, Monsarrat B, Bascands JL, Schanstra JP. Urine in clinical proteomics. *Mol Cell Proteomics* 2008; 7: 1850-1862.
- [26] Matheson A, Willcox MD, Flanagan J, Walsh BJ. Urinary biomarkers involved in type 2 diabetes: a review. *Diabetes Metab Res Rev* 2010; 26: 150-171.
- [27] Goodwill AG, Frisbee JC. Oxidant stress and skeletal muscle microvasculopathy in the metabolic syndrome. *Vascul Pharmacol* 2012; 57: 150-159.
- [28] Schaffer SW, Jong CJ, Mozaffari M. Role of oxidative stress in diabetes-mediated vascular dysfunction: Unifying hypothesis of diabetes revisited. *Vascul Pharmacol* 2012; 57: 139-149.
- [29] Lapolla A, Fedele D, Traldi P. Glyco-oxidation in diabetes and related diseases. *Clin Chim Acta* 2005; 357: 236-250.
- [30] Khechai F, Ollivier V, Bridey F, Amar M, Hakim J, de Prost D. Effect of advanced glycation end product-modified albumin on tissue factor expression by monocytes. *Arterioscler Thromb Vasc Biol* 1997; 17: 2885-2890.