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RESEARCH PAPER

Simultaneous quantification of endogenous and exogenous plasma glucose by isotope dilution LC-MS/MS with indirect MRM of the derivative tag

Lingling Yu[1,2] · Chao Wen[1,3] · Xing Li[1,3] · Shiqi Fang[1,3] · Lichuan Yang[4] · Tony Wang[4] · Kaifeng Hu[1]

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Abstract

Quantification of endogenous and exogenous plasma glucose can help more comprehensively evaluate the glucose metabolic status. A ratio-based approach using isotope dilution liquid chromatography tandem mass spectrometry (ID LC-MS/MS) with indirect multiple reaction monitoring (MRM) of the derivative tag was developed to simultaneously quantify endo-/exogenous plasma glucose. Using diluted D-[$^{13}C_6$] glucose as tracer of exogenous glucose, $^{12}C_6$/$^{13}C_6$ glucoses were first derivatized and then data were acquired in MRM mode. The metabolism of exogenous glucose can be tracked and the concentration ratio of endo/exo-genous glucose can be measured by calculating the endo-/exo-genous glucose concentrations from peak area ratio of specific daughter ions. Joint application of selective derivatization and MRM analysis not only improves the sensitivity but also minimizes the interference from the background of plasma, which warrants the accuracy and reproducibility. Good agreement between the theoretical and calculated concentration ratios was obtained with a linear correlation coefficient (R) of 0.9969 in the range of D-glucose from 0.5 to 20.0 mM, which covers the healthy and diabetic physiological scenarios. Satisfactory reproducibility was obtained by evaluation of the intra- and inter-day precisions with relative standard deviations (RSDs) less than 5.16%, and relative recoveries of 85.96 to 95.92% were obtained at low, medium, and high concentration, respectively. The method was successfully applied to simultaneous determination of the endo-/exogenous glucose concentration in plasma of non-diabetic and type II diabetic cynomolgus monkeys.

Keywords Derivatization · Diabetes · Endogenous glucose · Exogenous glucose · LC-MS/MS

Introduction

Monitoring glucose levels can provide objective evidence for the diagnosis and prevention of diabetes, hypertension, and cardiovascular and cerebrovascular diseases [1, 2]. Over the past decades, the methods reported for blood glucose detection include glucose oxidase-peroxidase (GOD-POD) [3], hexokinase (HK) [4], glucose dehydrogenase (GDH) [4], etc. These methods are fast and efficiently for determination of the overall blood glucose level, but they cannot distinguish between endogenous and exogenous glucose in plasma. The current assay established to simultaneously measure the endogenous and exogenous glucose levels is very critical for biomedical metabolic studies that involve the technique called glucose clamps, usually referring to hyperinsulinemic-euglycemic clamps, which are considered the "gold standard" for assessing whole body insulin sensitivity [5, 6]. When used in combination with tracer dilution techniques and physiological insulin concentrations, the insulin sensitization can be dissected and attributed to hepatic and peripheral effects that provide further details of metabolic status in body for evaluation of glucose turnover [7].

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✉ Kaifeng Hu
kaifenghu@mail.kib.ac.cn

[1] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, 132 Lanhei Road, Heilongtan, Kunming, Yunnan 650201, China

[2] School of Chemical Science and Technology, Yunnan University, Kunming, Yunnan 650091, China

[3] University of Chinese Academy of Sciences, Beijing 100049, China

[4] Kunming Biomed International, Kunming, Yunnan 650500, China

Previously, the analysis of glucose and other monosaccharide compounds by LC-MS was based on the formation of Na^+, NH_4^+, Cs^+ adducts in positive ionization mode and Cl^-, I^- adducts in negative ionization mode [8–13]. For glucose in positive ionization mode, inclination to form a variety of ion states, such as H^+, Na^+ and NH_4^+ adducts, and generation of multiple fragment ions due to the in-source collision induced dissociation (ISCID), make it difficult to obtain the accurate summation of these peak areas and thus accurate quantification. For example, monitoring NH_4^+ attachment cannot completely remove the interference from Na^+, which is a ubiquitous contaminant from glassware [11]. On the other hand, in negative ionization mode, Cl^- adducts have a major disadvantage of reducing sensitivity due to the presence of two natural isotopes of Cl^- [14–16]. Furthermore, the sensitivity of all these methods in positive or negative ionization mode is limited by the low ionization efficiency of the neutral carbohydrates.

Stable isotope-labeled glucose is often used as tracer to gain insight into glucometabolism. The tracer of [6,6-$^2H_2$] glucose, prepared in derivatized form, can be analyzed by gas chromatography mass spectrometry (GC-MS). However, this test is usually quite expensive because nearly 2 g of labeled material is needed [17]. Using [1-^{13}C] glucose as tracer, gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) offers superior analytical precision when compared with the conventional methodology of GC-MS and deuterium labeling [18]. The amount of tracer used can be reduced considerably, and so can the cost [17]. Similarly, using D-[$^{13}C_6$] glucose as tracer, analysis of isotopic enrichment of glucose in human plasma using liquid chromatography-isotope ratio mass spectrometry (LC-IRMS) has also been developed [19]. For IRMS-based methods (GC-C-IRMS and LC-IRMS), organic components eluting from a GC or LC column are oxidized in a capillary reactor (GC-C-IRMS) or by wet oxidation with peroxodisulfate under acidic conditions (LC-IRMS). The produced CO_2 is introduced into the isotope ratio mass spectrometer (IRMS), where the $^{13}C/^{12}C$ ratio was measured to determine the ^{13}C-isotopic enrichment in plasma samples, based on the peak areas of the *m/z* 44, 45, 46 signals of the separated compound [19]. IRMS-based methods (GC-C-IRMS and LC-IRMS) in fact only directly determine the $^{13}C/^{12}C$ ratio. The tracer/tracee ratio (labeled and natural glucose) is subsequently indirectly derived. The robustness of IRMS-based methods for the tracer/tracee ratio measurement heavily depends on performance of the chromatographic separation of the target compound. It is critical to prevent any ^{12}C or ^{13}C containing material from interfering with the analyte (glucose).

Isotope dilution mass spectrometry (IDMS) is a powerful tool for quantitative analysis of trace substances in complex matrices [20]. Using isotope dilution LC-MS to quantitatively analyze monosaccharide is drawing an increasing number of researchers' attention, which is becoming a mature technology in research and medical laboratory science [21]. For IDMS, peak area ratio of the fragment ions could be affected by the isotope effect, such as, for $^{12}C_6/^{13}C_6$ glucose when ^{13}C or ^{12}C is involved in the fragmentation (bond breaking). Derivatization method is often used to improve the ionization efficiency and sensitivity. Carbohydrate compounds can commonly be derivatized through reductive amination [22–25]. Glucose and other reductive sugars can react with amines generating Schiff base, which can be further reduced by reducing agents, such as sodium borohydride and sodium cyanoborohydride, giving the final stable derivative products [26, 27]. Derivatization of carbohydrates can significantly improve the ionization efficiency and the sensitivity of ESI.

Here, we report a new ratio-based approach using D-[$^{13}C_6$] glucose isotope dilution LC-MS/MS with indirect MRM of the dissociation of the derivative tag to accurately and simultaneously quantify endogenous and exogenous glucose in plasma. D-glucose and D-[$^{13}C_6$] glucose may have different probability and pattern of collision-induced dissociation (CID) due to the isotope effect if direct MRM of $^{12}C_6/^{13}C_6$ glucose is carried out without derivatization. In such case, the ratio of peak areas of the fragment ions of $^{12}C_6/^{13}C_6$ glucose may not correctly represent their quantity ratio. After derivatization reaction, induced by the collision the derivative tag is more easily dissociated (Fig. 1). This kind of indirect MRM of the derivative tag generates response-stable fragment ions and can effectively minimize the isotope effect. The peak areas of the extracted ion chromatograms (EIC) of the specific daughter ions at *m/z* 284 and *m/z* 290 were selected for the quantitative analysis. Compared to the methods mentioned above (GC-MS, GC-C-IRMS and LC-IRMS), the proposed LC-MS/MS is satisfactorily sensitive and more robust with extremely high specificity through selective derivatization and MRM analysis by dual selection of both the precursor and the daughter ions. In summary, the proposed method has the following advantages: (a) Joint application of selective derivatization and indirect MRM analysis warranted the sensitivity and specificity. (b) Significant reduction in the required quantity of labeled glucose brought clear cost advantage. (c) Validation results indicated that this method was robust with satisfactory accuracy and precision and can be used to simultaneously quantify endo/exo-genous plasma glucose.

Material and methods

Chemicals and reagents

Chromatographic grade methanol and acetonitrile (ACN) were obtained from Merck (Darmstadt, Germany). Milli-Q water was prepared by Millipore system (Merck Millipore, Germany). Glucose (purity 99.8%) was obtained from

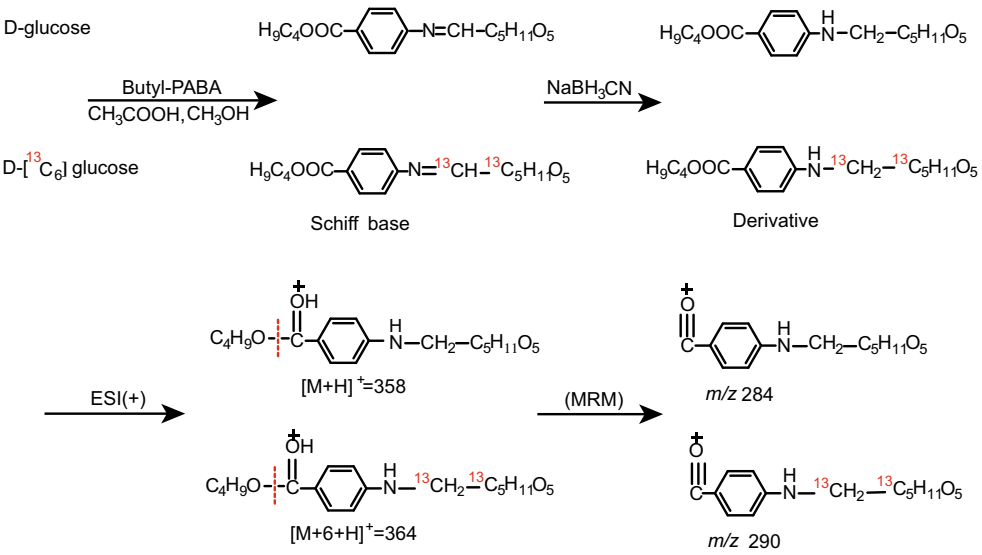

Fig. 1 The derivatization reaction and MS/MS analysis of D-glucose and D-[$^{13}C_6$] glucose

Solarbio Science and Technology Co., Ltd. (Beijing, China) and D-[$^{13}C_6$] glucose (U-$^{13}C_6$, isotope abundance 99%, purity 98%) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Derivatization reagents butyl-*p*-aminobenzoate (butyl-PABA) and sodium cyanoborohydride were purchased from Aladdin Industrial Corporation (Shanghai, China). Glacial acetic acid was purchased from Fengchuan Chemical Reagent Science and Technology Co., Ltd. (Tianjin, China). Formic acid (FA, 50%) and electrospray calibrant solution were purchased from Fluka (Switzerland). Derivatization reagent was prepared by mixing 0.385 g butyl-PABA, 0.625 g sodium cyanoborohydride, 0.375 mL glacial acetic acid, and 4.63 mL methanol [28].

Research participants and sample preparation

Plasma samples were obtained from non-diabetic and type II diabetes cynomolgus monkeys (For the Research participants, see Electronic Supplementary Material (ESM)). Two i.v. canules were inserted into the left and right forearms, one for each (left and right) forearm, one side is for the administration of the tracer substrates, and the other is for drawing blood samples. The first two blood samples (0.5 mL) were drawn into blood collection tubes at 10 and 3 min before the tracer infusion. Then the tracer (D-[$^{13}C_6$] glucose, prepared in normal saline in a concentration of 35 μmol/mL) was infused to the monkey with rates of 0.25 μmol/kg/min for the first hour and 0.1875 μmol/kg/min for the following 2 h. Blood samples (0.5 mL) were collected at 60, 75, 90, 105, 120, 135, 150, 165, and 180 min after the start of infusion. Plasma samples were prepared by centrifuging at 2000×*g* at 4 °C for 15 min and stored in − 80 °C freezer before tracer analysis.

Plasma samples were thawed at room temperature and vortex-mixed. A 100-μL aliquot was transferred to 1.5 mL microcentrifuge tube. Plasma proteins were precipitated with 400 μL acetonitrile followed by vortex-mixing for 30 s and centrifuged for 10 min at 12,000×*g* under 4 °C. Thereafter, the supernatant was transferred to 1.5 mL microcentrifuge tube and evaporated by Concentrator Plus (Eppendorf, Germany). The dried extracts were reconstituted in 100 μL water and dissolved fully.

Derivatization reaction and LC-MS/MS data collection

Twenty microliters of the above reconstituted sample was taken, and 100 μL of the derivatization reagent was added [28]. Derivatization reaction was shown in Fig. 1. After the reaction, the derivatization reaction mixtures were transferred to solid phase extraction (SPE) cartridges (C18/100 mg/1 mL, Germany) for sample pretreatment. Beforehand, the SPE cartridges were conditioned with 1 mL methanol and then pretreated with 1 mL water, and then equilibrated with 0.2 mL acetonitrile-water (*V*/*V*, 5:95) before run. The cartridges loaded with samples were washed twice with 1 mL of acetonitrile-water (*V*/*V*, 5:95) and then eluted with 1 mL acetonitrile-water (*V*/*V*, 30:70). The eluates were stored at 4 °C before LC-MS/MS data collection.

LC-MS/MS data were acquired on a UPLC system (Agilent 1290 infinity, USA) coupled with quadrupole time-of-flight tandem mass spectrometer (MicrOTOF Q II, Bruker Daltonics, Germany). A Waters CORTECS® UPLC® C 18 column (2.1 × 150 mm, 1.6 μm; Waters, Ireland) with a 2.1 × 5 mm guard column of the same material was used. Quadrupole tandem time-of-flight mass spectrometer was operated in positive ion mode with ESI interface and data acquisition done in the MRM mode. The capillary voltage was set at − 3000 V with an end plate offset potential of − 500 V. The scan range covered from 50 to 500 Da with an acquisition rate of 2.0 Hz. The dry gas (Nitrogen) was set to 3.0 L/min at

180 °C with a nebulization gas pressure of 0.8 bar. The mass axis was calibrated using the electrospray calibrant solution.

Analytical validation

Nine standard mixtures containing varying concentration of natural glucose (from 0.5 to 20.0 mM) and D-[$^{13}C_6$]-labeled glucose at fixed concentration of 0.035 mM were prepared to construct standard curve by linearly regressing the theoretical molar concentration ratios (x) with the calculated concentration ratios (y). Stock solutions of 100 mM D-glucose and 1 mM D-[$^{13}C_6$] glucose (concentration adjusted considering the purity level) were prepared in water. Different volumes of 100 mM D-glucose (10, 20, 40, 80, 140, 200, 280, 340, and 400 μL, respectively) were mixed with 70 μL of 1 mM D-[$^{13}C_6$] glucose, and were further diluted with water to a total volume of 2 mL, giving standard solutions of natural/labeled glucose mixture with D-glucose at the final concentrations of 0.5, 1.0, 2.0, 4.0, 7.0, 10.0, 14.0, 17.0, and 20.0 mM, respectively, and D-[$^{13}C_6$] glucose at the final concentration of 0.035 mM.

The accuracy and precision of the proposed method were assessed by the recoveries and intra- and inter-day RSDs. The recoveries were the measured percentage of standard glucose at three different concentration levels of 1 mM (low), 4 mM (medium), and 8 mM (high) spiked in plasma samples with D-[$^{13}C_6$] glucose as internal standard at fixed concentration of 0.035 mM (as matrix solution). The intra-day RSDs were calculated from six repeating analyses within the same day, and the inter-day RSDs were obtained from the measurements on three continuous days. The robustness of the assay was assessed on the samples above with glucose spiked at low, medium, and high concentrations by triplicate analyses using acetonitrile-water and methanol-water as eluting solution, respectively.

Results and discussion

Optimization of the experimental condition

In the initial study, the conditions of chromatography and collision energy have been optimized. Chromatographic performance of glucose derivative on Agilent ZORBAX Hilic Plus (2.1 × 150 mm, 1.8 μm) column was compared with that on a Waters CORTECS® UPLC® C18 (2.1 × 150 mm, 1.6 μm) column. The latter gave satisfactory separation after the mobile phase optimization. Thus, the CORTECS® UPLC® C18 column (2.1 × 150 mm, 1.6 μm) with a 2.1 × 5 mm guard column of the same material was used for chromatographic separation. The system was equilibrated with 30% solvent B (ACN with 0.1% (*V*/*V*) formic acid) and 70% solvent A (water with 0.1% (*V*/*V*) formic acid) for 3.0 min for the initial condition. For each run, 6 μL of the sample was injected and eluted at a flow rate of 0.20 mL/min with a linear gradient between 0 and 4.5 min, with solvent A and solvent B varying from 30 to 80% of B. The mobile phase was the held at 100% B for 4.5–7.0 min, and afterward changed back to the initial condition with 30% B and maintained for 3.0 min for system reequilibration. The total run time was 10.0 min including equilibration. A single sharp peak demonstrated an excellent retention and separation of glucose derivative, as shown in Fig. 2 0.1% formic acid added in the mobile phase can effectively facilitate the ionization efficiency. For MS/MS data acquisition, precursor ions at *m/z* 358 and *m/z* 364 were selected in MRM mode and collision energy was optimized in the range of 5.0 to 30.0 eV to maximize the intensity of the target specific daughter ions at *m/z* 284 and *m/z* 290, obtaining the most appropriate collision energy of 10.0 eV (see ESM Fig. S1).

Data analysis

The acquired data was processed using Compass DataAnalysis software (Bruker, Version 4.1). The extracted ion chromatograms (EIC) at *m/z* 284.11 and *m/z* 290.13 were extracted with extraction window width of ± 0.05 Da. The EIC peaks were smoothed using Gauss algorithm with a smoothing width of 1 point. The areas of chromatographic peaks corresponding to the derivatives of the $^{12}C_6/^{13}C_6$ glucose in EIC were manually integrated. Considering the natural abundance of ^{13}C (1.1%), ^{18}O (0.2%), ^{2}H (0.015%), ^{15}N (0.37%) and isotope labeling level of D-[$^{13}C_6$] glucose (U-$^{13}C_6$, isotope abundance 99%), the obtained EIC chromatographic peak areas A_{284} (or $A_{[M]}$) and A_{290} (or $A_{[M+6]}$) can be translated into the concentration ratio between the endogenous (natural) and exogenous (D-[$^{13}C_6$] labeled) glucose:

$$\frac{C_{endo}}{C_{exo}} = \frac{C_N}{C_L} = \frac{A_{284}/0.8123}{A_{290}/0.8200} \quad (1)$$

The A_{284} (or $A_{[M]}$) and A_{290} (or $A_{[M+6]}$) are the peak areas in EIC at *m/z* 284.11 ± 0.05 and *m/z* 290.13 ± 0.05, which are the specific fragment ions of the derivatives of D-glucose and

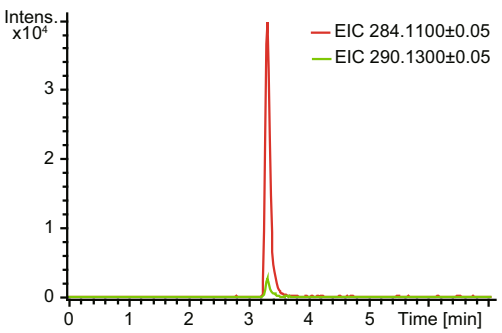

Fig. 2 EIC chromatogram derived from the standard mixture solution of 0.5 mM D-glucose and 0.035 mM D-[$^{13}C_6$] glucose

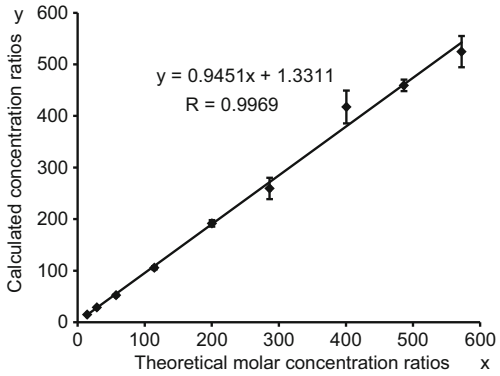

Fig. 3 Linear regression of the theoretical molar concentration ratios (x) with the calculated concentration ratios (y) between natural D-glucose and D-[$^{13}C_6$] glucose derivatives. Vertical error bars indicate the standard deviation of triplicate measurements at each point

D-[$^{13}C_6$] glucose. C_{endo} (or C_N) and C_{exo} (or C_L) represent the calculated concentrations of endogenous (natural) and exogenous (D-[$^{13}C_6$] labeled) glucose.

Method validation

The assay was validated for linearity, accuracy, precision, and robustness. The concentration ratios (y) between natural and labeled glucose are calculated from the measured LC-MS/MS peak intensities in EIC at m/z 284 and m/z 290 following Eq. (1) above. Satisfactory agreement between the theoretical and calculated concentration ratios was obtained. Linear regression of the theoretical molar concentration (adjusted by the purity level) ratios (x) with the calculated concentration ratios (y) results a correlation coefficient $R = 0.9969$ with the D-glucose dynamic range of 0.5–20.0 mM (corresponding to the concentration ratio of 14.31–572.35), which covers the healthy and diabetic physiologic scenarios (Fig. 3). The standard deviations (SD) were estimated from triplicate data at each different concentration ratio. The relative errors (RE%) at each different concentration ratio were calculated using the average values of the triplicate measurements against the theoretical ones, which are all within ± 10% (Table 1).

The ratio of D-glucose and D-[$^{13}C_6$] glucose for the matrix solution was calculated following Eq. (1), which is then subtracted from those calculated for the plasma samples spiked with different levels of D-glucose. The relative recoveries were then calculated as the difference divided by the theoretical ratios of the spiked materials (Eq. (2)).

$$\text{Recovery}\% = \frac{C_N/C_L(\text{spiked}) - C_N/C_L(\text{matrix})}{C_N/C_L(\text{ theo})} \quad (2)$$

As shown in Table 2, relative recoveries ranging from 85.96 to 95.92% and acceptable precision with RSDs values of less than 5.16% were achieved, demonstrating satisfactory accuracy and reproducibility of the proposed method.

The robustness of the proposed ratio-based approach using isotope dilution LC-MS/MS with indirect MRM of the derivative tag was evaluated from RSDs ranging from 2.73 to 5.33% under different chromatographic conditions with regard to glucose at low, medium, and high concentrations (Table 3). These results demonstrate that changes of chromatographic conditions did not influence significantly the analytical results.

Table 2 Accuracy and precision (intra- and inter-day) for the determination of D-glucose in plasma

	Low (1 mM)	Medium (4 mM)	High (8 mM)
Intra-day precision (RSDs%, $n = 6$)	3.09	2.40	3.82
Inter-day precision (RSDs%, $n = 3$)	3.73	4.57	5.16
Recovery (%)[b]	95.92 ± 7.65	86.20 ± 2.97	85.96 ± 4.02

[b] Mean ± SD, $n = 6$

Table 1 Statistical analyses and linearity of the measurements on the standard mixtures

	Molar concentration (mM)		Theoretical ratios	Calculated ratios	RE%
No.	D-glucose	D-[$^{13}C_6$] glucose	C(^{12}C)/C(^{13}C)	A(^{12}C)/A(^{13}C)[a]	($n = 3$)
1	0.5	0.035	14.31	14.83 ± 0.55	3.65
2	1.0	0.035	28.62	28.76 ± 1.06	0.51
3	2.0	0.035	57.23	52.52 ± 1.94	− 8.24
4	4.0	0.035	114.47	105.57 ± 2.93	− 7.78
5	7.0	0.035	200.32	191.76 ± 6.16	− 4.27
6	10.0	0.035	286.17	259.34 ± 20.70	− 9.38
7	14.0	0.035	400.64	417.42 ± 31.19	4.19
8	17.0	0.035	486.49	459.26 ± 11.15	− 5.60
9	20.0	0.035	572.35	524.56 ± 30.25	− 8.35

[a] Mean ± SD, $n = 3$

Table 3 The robustness of proposed method

	Low (1 mM)	Medium (4 mM)	High (8 mM)
ACN-H_2O (0.1%FA) (RSDs%, $n = 3$)	1.61	2.15	4.45
CH_3OH-H_2O (0.1%FA) (RSDs%, $n = 3$)	3.40	3.82	5.94
ACN-H_2O (0.1%FA) + CH_3OH-H_2O (0.1%FA) (RSDs%, $n = 6$)	2.73	2.80	5.33

Using an ESI ion source, the major limitation is variable ionization efficiency due to the matrix effect, which prevents constructing a direct linear relation between the concentration and the measured MS peak intensity. As shown in Fig. S2 (see ESM), with the gradually increasing concentration of D-glucose derivative, ionization efficiency of both the labeled and non-labeled glucose derivative decreases, compared to the peak intensities predicted based on the intensity at the lowest concentration (dashed lines). The infused D-[$^{13}C_6$] glucose (as the tracer of the exogenous glucose) can meanwhile be regarded as isotopic internal standard for the endogenous glucose for quantitative analysis, in which case the concentration of the endogenous and exogenous glucose can be respectively and accurately measured with the MS matrix effect of the plasma samples compensated [29]. The peak areas of the daughter ions (*m/z* 284, 290) of the derivatives were used to calculate the ratio of endogenous and exogenous glucose following Eq. (1). In such a case, though existing, the matrix effect does not change the final concentration ratio [30], which is proportional to the ratio of peak intensities derived from the labeled and unlabeled glucose. After derivatization, collision-induced dissociation (CID) of the common unlabeled derivative tag (indirect MRM) of $^{12}C_6/^{13}C_6$ glucose has ignorable isotope effect, which further improves the measurement accuracy of the concentrations of the endogenous and exogenous glucose in plasma.

Determination of endogenous and exogenous plasma glucose concentration

Using D-[$^{13}C_6$] glucose as a tracer of exogenous glucose can accurately evaluate the glucose metabolic status of the organism, especially for individuals with diabetes and insulin resistance. In order to illustrate the biomedical application of this method, as described in Section "Research participants and sample preparation," D-[$^{13}C_6$] glucose was continuously infused in non-diabetic and type II diabetic cynomolgus monkeys and plasma samples were collected before and after the start of infusion. The dried extracts of plasma samples were reconstituted in 100 μL water for the following derivatization reaction and LC-MS/MS data acquisition. Provided the concentration of the overall (endogenous and exogenous) plasma glucose is known (can be measured by conventional way, such as, by the UV absorbance after oxidation), the concentrations of endogenous and exogenous glucose in plasma can be accurately and respectively determined based on their ratio derived from Eq. (1).

As shown in Fig. 4a, at the same glucose infusion rate, the concentration of the exogenous D-[$^{13}C_6$] glucose in the plasma of non-diabetic subject was lower than that of diabetic subject, indicating that the glucose utilization rate by the peripheral musculature of diabetic subjects was relatively slower than that of the non-diabetic subjects until a higher hemeostatic concentration of the exogenous glucose is reached. Concomitantly, the insulin concentrations of diabetic subject are compensatorily higher (see ESM Fig. S3). Previous studies on people with type II diabetes indicated that hyperglycemia and hyperinsulinemia were both potent inhibitors of glycogenolysis [31]. With the infusion of the exogenous D-[$^{13}C_6$] glucose, the level of endogenous glucose decreases, indicating the levels of glycogenolysis and gluconeogenesis continued to decline for both non-diabetic and diabetic subjects. Further, comparing the endogenous glucose turnover data of the non-diabetic and type II diabetic monkeys, it was evident that diabetic subject has a faster and greater decrease in endogenous glucose than

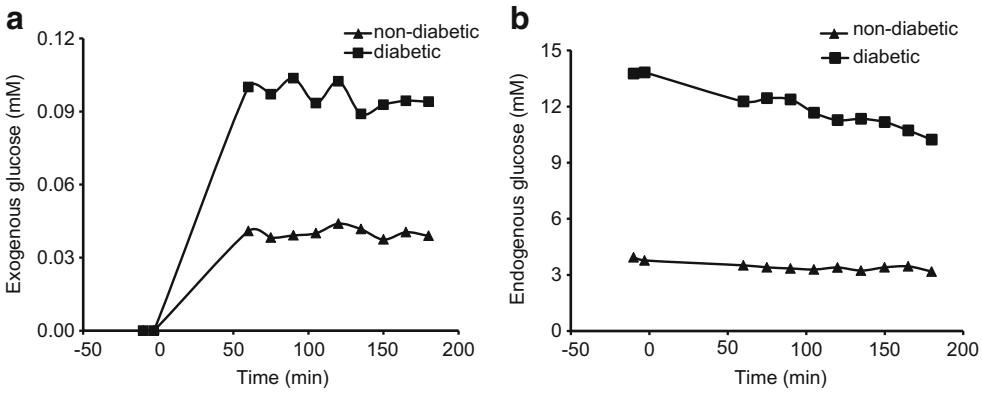

Fig. 4 The exogenous (**a**) and endogenous (**b**) glucose levels in the plasma of non-diabetic and diabetic subjects at different time points

non-diabetic subject (Fig. 4b), which can be attributed to stronger insulin-induced suppression of glycogenolysis as well as gluconeogenesis of diabetic subject.

The described method can provide a ratio-based approach to accurate determination of endogenous and exogenous plasma glucose concentration in clinical studies of glucose kinetics in healthy and diabetic individuals, which can more comprehensively depict the glucose turnover and evaluate the glucose metabolic status of organisms, and is helpful for better understanding the pathology of diabetes.

Conclusions

We reported a robust, highly selective, sensitive and accurate LC-MS/MS method for simultaneous quantification of endogenous and exogenous glucose in plasma from non-diabetic and diabetic cynomolgus monkey. Dissection of the endogenous and exogenous plasma glucose can provide more detailed information on the glucose kinetics, which will be helpful for better evaluating the glucose metabolic status of organisms and understanding the pathology of diabetes. Glucose selective derivatization together with specific precursor-daughter ion pairs selected in MRM mode warrants extremely high selectivity of this method. Therefore, it can be used to accurately quantify glucose in the complex background of plasma with minimal influence, even in existence of considerable amount of isobaric interferences (at *m/z* 364). Meanwhile, derivative of glucose has much higher ionization efficiency than the unmodified glucose, which can thus dramatically improve the sensitivity. Double selection of the specific precursor (*m/z* 358, 364) and specific daughter (*m/z* 284, 290) ions gives much cleaner background, which can further improve the signal-to-noise ratio.

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Compliance with ethical standards All protocols of animal experiments were approved by the Institutional Animal Care and Use Committees at Kunming Biomed International.

Conflict of interest The authors declare that they have no conflicts of interest.

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