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Quantitation of Monosaccharide Isotopic Enrichment in Physiologic Fluids by Electron Ionization or Negative Chemical Ionization GC/MS Using Di-*O*-isopropylidene Derivatives

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The aldonitrile pentaacetate and other derivatives lack ions in the electron ionization (EI) spectra possessing an intact hexose structure and thus must be analyzed by chemical ionization GC/MS in order to study multiple isotopomers. We report methods for quantitation of hexose di-O-isopropylidene acetate (IPAc) or pentafluorobenzoyl (PFBz) esters. These were prepared in a two-step procedure using inexpensive reagents that do not adversely impact the isotopomer structure of the sugar. The acetate derivative possesses an abundant [M − CH_3] ion in the EI spectrum which is suitable for quantitative analysis of isotopomers. The negative chemical ionization (NCI) spectrum of the corresponding pentafluorobenzoyl derivative has a dominant molecular anion. Moreover, the PFBz derivative is about 100-fold more sensitive than the acetate, which offers some advantages for analysis of minor hexoses found in plasma. Isotopic calibration curves of [U-^{13}C]glucose are linear over the 0.1−60% tracer/tracee range tested. The useful range for isotopic tracer studies is 25−2500 pmol for EI analysis of the acetate derivative and 0.1−55 pmol for NCI analysis of PFBz derivative (sample amount injected). For most studies where sample size is not limited, EI-GC/MS analysis of the IPAc derivative is preferred. NCI-GC/MS analysis is reserved when sample size is limiting or when studies involve hexoses other than glucose that are normally present at low concentration.

Analysis of the stable isotopic enrichment in plasma glucose is one of the more enduring procedures in physiology and metabolism. These measurements are used to determine rates of hepatic glucose output in a variety of clinical conditions.[1−11] More recently, investigators have focused their attention on quantifying the enrichment of multiple isotopomers of glucose derived from isotopic infusions of [2-^{13}C]alanine, [2-^{13}C]glycerol, and [U-^{13}C]-glucose to estimate the relative contribution of gluconeogenesis to glucose production.[3,6,12−18]

Several techniques and derivatives have been described to quantitate glucose isotopic enrichment. The more common methods use the pentaacetate,[6,19,20] aldonitrile pentaacetate,[12,19,21−23] methyloxime pentatrimethylsilyl,[1,12,24] bisbutylboronate acetate,[12,25,26] and permethyl derivatives.[12,27] Although each derivative has its merits, those containing silicon or boron introduce significant isotopic complexity in the mass spectrum, which limits their usefulness for quantitation of isotopomers present at low enrichment. The aldonitrile pentaacetate, simple hexose pentaacetates, and permethylated hexoses generally lack ions in the electron ionization (EI) spectrum possessing an intact hexose structure and thus must be analyzed by chemical ionization (CI)-GC/MS in order to study the complete set of isotopomers.

The classic work by Biemann firmly established the utility of MS for structural analysis of pentose and hexose acetates[20] and di-*O*-isopropylidene[28] derivatives. Despite the fact that the *O*-isopropylidene derivatives have been known for more than 60

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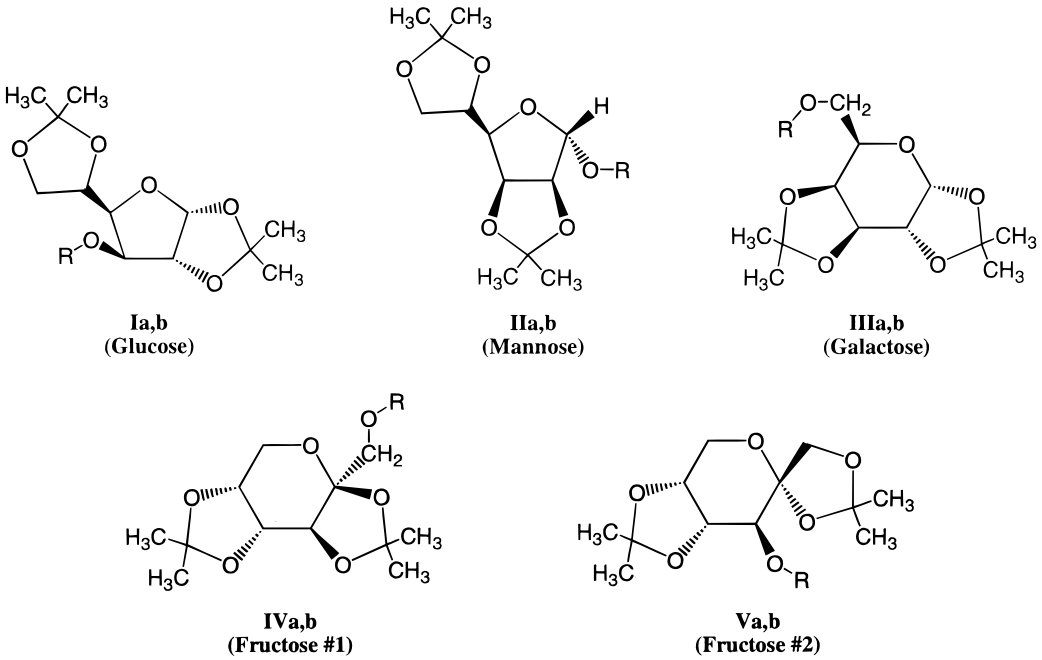

Figure 1. Structures of the major hexose derivatives: 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**Ia**,**b**), 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose (**IIa**,**b**), 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**IIIa**,**b**), 2,3:4,5-di-*O*-isopropylidene-*β*-D-fructopyranose (**IVa**,**b**), 1,2:4,5-di-*O*-isopropylidene-α-D-fructopyranose (**Va**,**b**).

years, possess favorable EI spectra and good chromatographic properties, and are easily prepared in high yield using inexpensive reagents, they have received scant attention from the biomedical community.[29,30] We report here a simple procedure for isolation of glucose from plasma, preparation of di-*O*-isopropylidene derivatives, and analysis of the acetylated derivatives by electron ionization GC/MS. Using a simple modification of the final derivatization step, the procedure can be adapted to microscale analysis of serum monosaccharides as the di-O-isopropylidene pentafluorobenzoyl ester with isotopic analysis by negative chemical ionization (NCI)-GC/MS.

MATERIALS AND METHODS

[U-^{13}C]Glucose and [6,6-$^{2}H_2$]glucose (99 atom %) were obtained from Cambridge Isotope Laboratories (Woburn, MA). D-Glucose, D-galactose, D-fructose, D-mannose, and D-xylose and derivatization reagents were obtained from Sigma-Aldrich (St. Louis, MO). Reference standards of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose were obtained from Aldrich Chemical Co. (Milwaukee, WI). [^{14}C]Glucose was obtained from CEA (Gif-sur-Yvette, France). Capillary GC columns were obtained from Hewlett-Packard (Palo Alto, CA) and Supelco (Bellefonte, PA). Common inorganic reagents and solvents were obtained from Fisher Scientific (Houston, TX). Stock solutions of labeled compounds and reference monosaccharides were prepared to a nominal concentration of 7.5 mM in 20% acetone/water to prevent microbial growth. Isotope dilution curves covering a tracer/tracee ratio of 0.001:1−0.60:1 were prepared by serial volumetric dilutions from these standards. Stock solutions were stored in the freezer at −20 °C until needed.

After informed consent was obtained, a glucose kinetic study was conducted in a 56-year-old, 103.4-kg male subject diagnosed with noninsulin-dependent diabetes mellitus. The subject received a priming bolus dose of 20.0 μmol·kg^{-1} and a constant infusion of 20.0 μmol·kg^{-1}·h^{-1} of [U-$^{13}C_6$]glucose for 6 h. Eight blood samples were collected over the duration of the study. The Institutional Review Board of Baylor College of Medicine approved this protocol.

Preparation of Di-*O*-isopropylidene Acetate (IPAc) Derivatives of Hexoses. The general derivatization chemistry is illustrated in Figure 1 for glucose. Approximately 1−5 μmol (180−1000 μg) of each carbohydrate was transferred to a 10-mL screw-cap culture tube and evaporated to dryness in vacuo. Ten microliters of water was added to dissolve the sugars, followed by addition of 1 mL of 0.38 M sulfuric acid in acetone. The samples were incubated at room temperature for 60 min and then carefully neutralized with 2 mL of 0.44 M sodium carbonate, followed by 2 mL of saturated sodium chloride. *O*-Isopropylidene derivatives were extracted twice with 3 mL of ethyl acetate, centrifuged, transferred to a 2-mL, vial and evaporated to dryness under nitrogen on a cold manifold. The O-isopropylidene derivatives were acetylated with 200 μL of ethyl acetate/acetic anhydride (1:1) at 60 °C for 30 min. Samples were diluted as necessary with ethyl acetate to give a working concentration of 125 μM (~25 ng/μL). The small amount of acetic anhydride present is harmless to nonpolar polysiloxane capillary GC columns.

Preparation of Di-*O*-isopropylidene Pentafluorobenzoyl (PFBz) Derivatives of Hexoses. Approximately 10−50 nmol (2−10 μg) of each carbohydrate was derivatized as just described, except for the final treatment with acetic ethyl acetate/anhydride. To form the PFBz derivative, the dried di-*O*-isopropylidene derivative was dissolved in 250 μL of anhydrous pyridine, followed

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by addition of 25 μL of pentafluorobenzoyl chloride. The sample was vortexed and then heated at 60 °C for 10 min. The PFBz derivatives were extracted with 3 mL of hexane and washed twice with 2 mL of water to remove pyridine and excess pentafluorobenzoyl chloride. The hexane layer was transferred to a 2-mL vial and evaporated to dryness under nitrogen. The samples were diluted as necessary with hexane to give a working concentration of 1 μM ($\sim$180 pg·μL^{-1}).

Preparation of Di-*O*-isopropylidene Derivatives of Plasma Glucose. Plasma (50−100 μL) was pipetted into a 1.5-mL microcentrifuge tube, followed by 1 mL of cold (0 °C) acetone. The tube was capped, vortexed, and centrifuged at 2000g for 5 min to remove plasma proteins. The acetone was decanted into a 10-mL Teflon-capped culture tube and evaporated to dryness under nitrogen. One milliliter of freshly prepared 0.38 M sulfuric acid in acetone was added, and the sample was incubated at room temperature for 60 min. At this point, either the acetate or the pentafluorobenzoyl esters can be prepared as described above. Note that for routine measurement of glucose isotopomer enrichment, the acetate derivative with analysis by EI-GC/MS is preferred. The pentafluorobenzoyl esters may require dilution with large volumes of solvent, so this method is reserved for studies of minor hexoses present at low concentration in plasma or when plasma sample size is limited.

Derivatization Efficiency. Laboratory control plasma and aqueous glucose standards were spiked with about 50 000 dpm·mL^{-1} [^{14}C]glucose. A 100-μL aliquot of each sample was subjected to the derivatization procedure just described. Aliquots were taken after the protein precipitation step and at the final derivatization step for radioactivity determination. Results were expressed as percent of the initial spike normalized to the volume of plasma analyzed.

GC/MS Analysis of Di-*O*-isopropylidene Acetate or Pentafluorobenzoyl Derivatives. Capillary GC/MS analysis was performed using a nonpolar HP-5ms 5% diphenyldimethylpolysiloxane column (30 m × 0.25 mm i.d., 0.25-μm phase thickness), a medium-polarity HP-1701 (cyanopropylphenyl)dimethylpolysiloxane column (20 m × 0.18 mm i.d., 0.25-μm phase thickness), or a SPB-17 50% (diphenyldimethyl)polysiloxane column (30 m × 0.25 mm i.d.). Samples were chromatographed using injector and detector temperatures of 250 °C, a helium inlet pressure of 6 psi, and splitless injection. The typical column temperature program was from 80 to 260 °C at 10 °C·min^{-1}, after a 1-min injection hold. Electron ionization (70 eV) mass spectra were acquired on either a Hewlett-Packard HP5970 mass-selective detector or a HP5989 MS Engine. NCI mass spectra were acquired on the HP5989A instrument. The ion source temperature was 200 °C, methane reagent gas pressure was typically 0.9 Torr, and electron energy was 150 eV. Full-scan spectra were acquired from m/z 20 to 550 at about 2 scans/s. Selected ion monitoring data were acquired using a 25-ms ion integration time. Instruments were tuned and calibrated in EI mode using the instrument "autotune" procedure. NCI tuning was done manually to achieve the best sensitivity, resolution, and ion peak shape. Calibration curves and quantitative standards were generally analyzed in triplicate. Biological samples were usually analyzed twice, depending on the level of enrichment.

Statistical and Numerical Methods. Statistical analysis of the tracer data was performed using Minitab Statistical Software Release 11.1 (Minitab, Inc., State College, PA). One-way analysis of variance (ANOVA) was done to determine mean, standard deviation, and pooled standard deviation. The measurement systems analysis subroutines in Minitab were used to evaluate within-sample and between-sample variance. Theoretical isotope ratios were computed using a copy of ISOPRO version 3.0 that was kindly supplied by Dr. Michael Senko of Finnigan MAT Corp. Numerical deconvolution of the tracer data was done as described by Hachey et al.[31] The correction matrix for glucose was computed from the mean tracer ratios of the two unenriched baseline samples using the CORMAT computer program described by Fernandez et al.[16] However, the [U-^{13}C]glucose entry in the matrix was obtained from a pure tracer standard. Dr. Henri Brunengraber of Case Western University was kind enough to provide a copy of CORMAT.

RESULTS AND DISCUSSION

Derivatization Procedures. Figure 1 shows the structures of di-*O*-isopropylidene esters formed by acetonation with 0.38 M sulfuric acid, followed by acylation with either acetic anhydride or pentafluorobenzoyl chloride. The mass spectra and fragmentation patterns of the acetate esters were identified by comparison to the hydroxy compounds published by Biemann[28] and Morgenlie[30] and the acetylated glucose derivative **Ia** reported by Gagnaire.[29] Acetylation improves and maintains the capillary GC column performance and peak shape, which tends to degrade with repeated analysis of large numbers of physiologic glucose samples. A small amount of water ($\sim$10 μL) was used to dissolve crystalline standards before acetonation due to the poor solubility of hexoses in anhydrous acetone. High concentrations of water generally resulted in poor yields of isopropylidene derivatives. To form stable acetone adducts a cis 1,2- or cis 1,3-diol conformer is required. Thus, various di-*O*-isopropylidene structures are possible depending on the initial orientation of the hydroxyl groups in the hexose. The composition of the final product is determined by thermodynamic factors caused by intramolecular steric interactions with the bulky *gem* dimethyl groups. Glucose, for example, forms predominantly the 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (Figure 1, **Ia,b**), with a trace amount of two other diacetone adducts, as noted also by Morgenlie.[30] Although **Ia** always dominates the mixture, the relative composition depends on the amount of water and the time allowed for derivatization. Galactose also forms a minor acetone adduct, but mannose forms exclusively the 2,3:5,6-adduct (Figure 1, **IIa**,**b**). Fructose forms a 2:1 mixture of 2,3:4,5- (Figure 1, **IVa**,**b**) and the 1,2:4,5-adducts (Figure 1, **Va**,**b**). Figure 2 shows the chromatographic separation of a standard mixture of pentose and hexose di-*O*-isopropylidene derivatives. Although the minor glucose derivatives are present at only a few percent of the total, they are generally resolved from other hexoses (Figure 2). However, they may interfere with quantitation of the minor hexoses due to the much greater concentration of glucose in plasma ($\sim$5.5 mM) than that of other hexoses. A chromatogram of the PFBz derivatives is shown in

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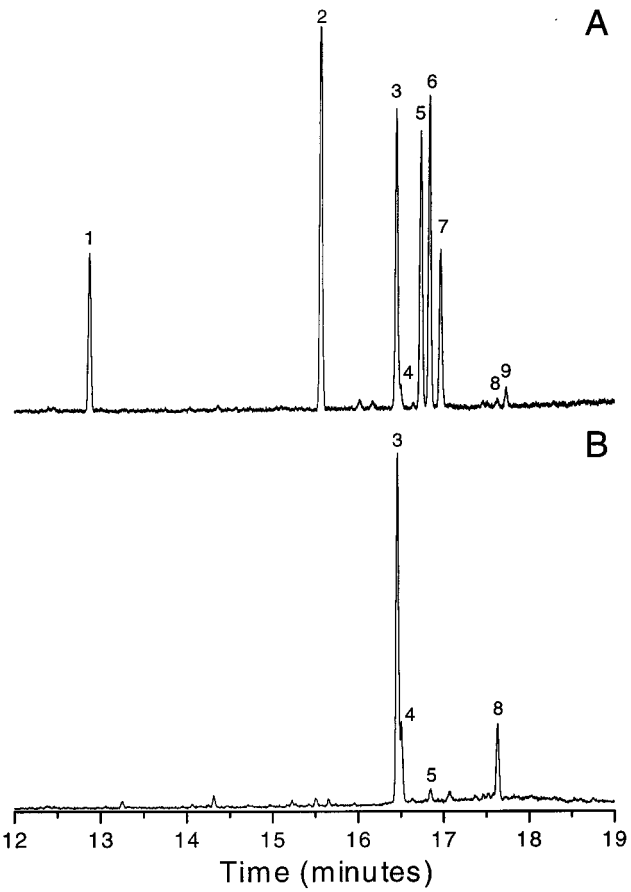

Figure 2. Total ion chromatogram of a mixture of IPAc derivatives (A) of xylose (1), fructose (2, **IVa**,**b**), glucose (3, **Ia**,**b**), a glucose byproduct (4), mannose (5, **IIa**,**b**), galactose (6, **IIIa**,**b**), fructose (7, **Va**,**b**), and minor glucose (8) and galactose (9) byproducts. Chromatogram B illustrates the typical composition of plasma hexoses in the fed state. Note the presence of a small amount of mannose at 16.9 min. See Figure 1 for a description of the structures.

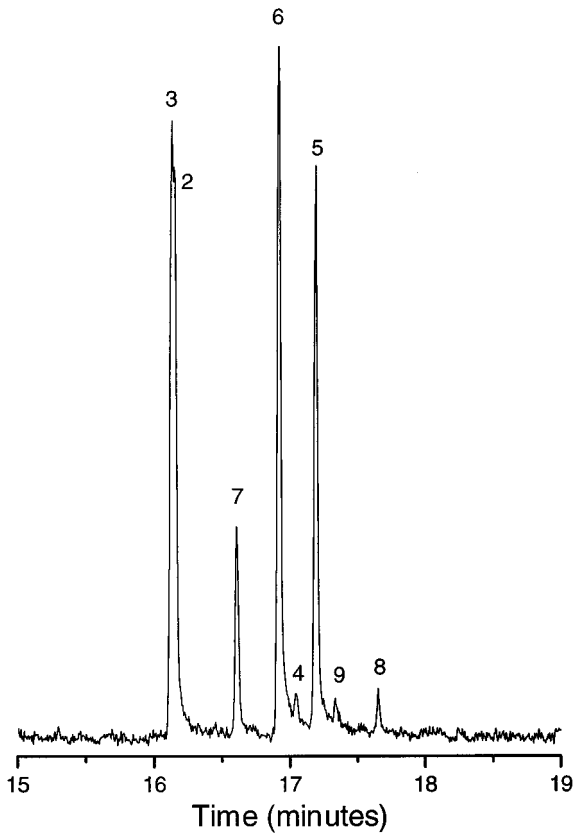

Figure 3. Total ion chromatogram of the PFBz derivatives of xylose and hexose standards. Peak identitification is described in Figure 3. Note the absence of a xylose peak. Di-*O*-isopropylidene xylofuranose, and other pentoses, lack a free hydroxyl and do not form PFBz derivatives. See Figure 1 for a description of the structures.

Figure 3. Separation of various hexoses is similar to that of the acetate, but glucose and fructose are not resolved on a 30-m 5% diphenyldimethylsiloxane column. Fructose and galactose concentration in plasma of healthy, fasting subjects is normally below detection limits.

Isolation and derivatization efficiency was determined using [^{14}C]glucose. Protein precipitation using acetone resulted in 60 ± 5% recovery in the supernatant, with the remainder bound to the protein pellet. Extraction of the final derivatized acetonide with hexane gave 22 ± 11% recovery, whereas extraction with ethyl acetate gave 44 ± 21% overall recovery. Traces of water tend to quench acetonide formation, so the acetone supernatant obtained in the protein precipitation should be dried carefully. Because the emphasis in this work is on isotope ratio determination and the concentration of glucose in plasma is sufficiently high, no further attempts to improve the derivatization were attempted. For quantitative analysis, the addition of a stable isotope labeled internal standard would overcome the somewhat low recovery.

Electron Ionization Mass Spectra. The 70-eV electron ionization mass spectra of the four major hexoses are shown in Figure 4. The corresponding acetates generally followed the fragmentation pathways described by Biemann[28] for the hydroxy di-*O*-isopropylidene derivatives. No molecular ions were observed. The base peak at m/z 43 is due to intramolecular rearrangement of the 1,3-dioxolane ring on the acetonide and ejection of a stable CH_3CO^+ cation. Although there are significant differences in fragmentation patterns between the pyranose and furanose structures, the ion at m/z 287, $[M - CH_3]^+$, is a common feature in all the hexoses. Because this ion is relatively abundant, is free of interfering fragments, and contains the intact hexose carbon backbone, it is useful for isotopic quantitation. Other analytically useful ions are observed at m/z 169 [M − 133], which retains $C_1 - C_6$; m/z 143 [M − 159], which retains $C_1 - C_4$; and m/z 101 [M − 201] caused by scission between C_4 and C_5, which retains $C_5 - C_6$.

Negative Chemical Ionization Mass Spectra. The methane NCI spectra contain a dominant molecular anion at m/z 454 $[M]^-$ caused by resonance capture of a thermal electron, as illustrated for the galactose derivative **IIIb** (Figure 5). Minor ions are present at m/z 396 $[M - CH_3COCH_3]^-$ and 380 [M − acetone − O]$^-$. In addition, there are ions at m/z 148, $C_6F_4^-$; m/z 167, $C_6F_5^-$; and m/z 195 $C_6HF_5CO^-$ from the PFBz group. The NCI spectrum of the other hexoses is virtually identical to that of **IIIb**, differing slightly in the relative abundance of the minor ions. The ion at m/z 454 is used for isotopic ratio measurements.

Isotope Calibration Curves. Isotope calibration curves were constructed using [U-$^{13}C_6$]glucose covering a tracer/tracee ratio of 0.001:1−0.60:1. Isotope dilution standards were analyzed in triplicate. The amount of sample injected on the column

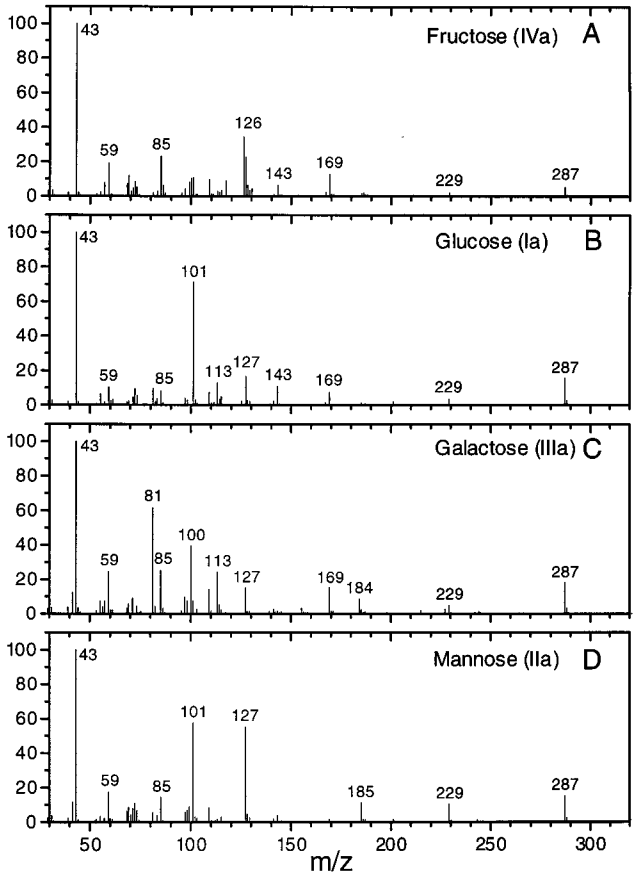

Figure 4. Electron ionization mass spectra of the hexose di-*O*-isopropylidene derivatives of (A) fructose (**IVa**), (B) glucose (**Ia**), (C) galactose (**IIIa**), and (D) mannose (**IIa**). See Figure 1 for a description of the structures.

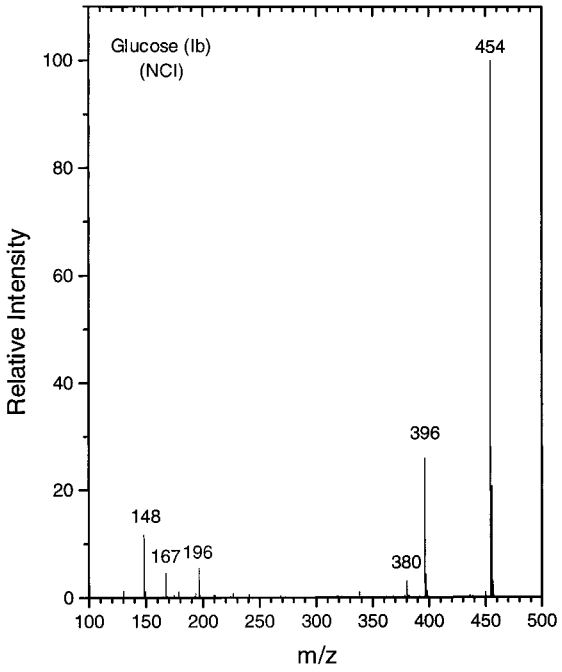

Figure 5. Methane NCI mass spectrum of 1,2:3,4-di-*O*-isopropylidene-6-pentafluorobenzoyl-α-D-galactopyranose (**IIIb**).

ranged from 5 to 500 ng for EI measurements and 50 to 5000 pg for NCI. Under these conditions, the curves were linear over this range of isotopic enrichment, and they did not show any significant analytical bias when different amounts of analyte were injected. The slopes of the lines were 0.994−1.049 (R = 0.999) for the EI data and 0.952−0.987 (R = 0.999) for the NCI data.

Glucose Infusion in a Diabetic Subject. To illustrate the suitability of these methods for analysis of tracer data from metabolic kinetic studies, a primed-constant infusion protocol with [U-$^{13}C_6$]glucose was conducted in a 56-year-old male subject with noninsulin-dependent diabetes mellitus. The mean ion ratios from triplicate determinations of the [M + 1] through [M + 6] isotopomers are summarized in Table 1. From the ion ratios, a corrected set of isotopomer data (Table 2) was computed by solving a set of simultaneous linear equations using standard matrix algebraic techniques.[31] The isotopic enrichment of the [M + 6] isotopomer represents that of the parent unmetabolized tracer, while that of the [M + 3] isotopomer reflects the primary metabolic cleavage into two three-carbon metabolites and reincorporation into glucose by endogenous hepatic biosynthesis. The [M +1] and [M + 2] isotopomers are derived from further metabolism of the three-carbon fragments, followed by reincorporation into glucose, while the [M + 4] and [M + 5] isotopomers are recombinant products of the [M + 3] isotopomer with either the [M + 1] or the [M + 2] isotopomer. The isotopic enrichment of the [M + 2] isotopomer is detectable, but with poor precision due to the low absolute infusion rate of tracer in this subject. The isotopic enrichment of the [M + 1] was not quantifiable due to the inherent difficulty of measuring a small enrichment on top of a large natural-abundance isotopomer. The [M + 4] and [M + 5] isotopomers were not detectable due to the low probability of two three-carbon unit recombination. Normally such studies are conducted at 10−30-fold greater infusion rates in order to achieve sufficient precision to be physiologically useful. In this example, the plateau isotopic enrichment of the [M + 6] isotopomer (0.011 29 ± 0.001 42) corresponds to a glucose flux of 1771 μmol·kg^{-1}·h^{-1} in the fed state. The precision of the assay for the [M + 6] isotopomer based on triplicate measurements was typically better than 3%. Of the total variance in the measurement system, the within-sample variance was 6.3%, which reflects the inherent instrument precision. Most of the variance is explained by sample-to-sample processing factors, even though the absolute error is quite low. These data are consistent with a heteroscedastic variance structure typically observed in stable isotope tracer studies.

Measurement of the enrichment of glucose isotopomers is most commonly done by methane positive chemical ionization in order to preserve intact the carbon skeleton of the molecule. Few glucose derivatives possess a favorable electron ionization fragmentation pattern except the bis-*n*-butylboronate derivative.[12,25,26] However, the unfavorable isotope distribution of boron limits the utility of this derivative for quantitation of multiple isotopomers. On the basis of the classical work by Biemann and his students on the electron ionization mass spectra of carbohydrates,[28] we expected the acetylated di-*O*-isopropylidene derivatives to possess a favorable EI spectrum. This derivative can be prepared rapidly and safely using inexpensive reagents; it has excellent thermal and chemical stability; and it affords good capillary GC performance. Moreover, the carbon burden of the

Table 1. Ion Abundance (×100) of Isotopomers in Plasma Glucose from a Subject Receiving a Constant Infusion of [U-$^{13}C_6$]Glucose

sample	time (h)	[M + 1]	[M + 2]	[M + 3]	[M + 4]	[M + 5]	[M + 6]
1	−0.50	15.541 ± 0.031	2.642 ± 0.011	0.297 ± 0.022	0.046 ± 0.012	nd	nd
2	−0.25	15.230 ± 0.113	2.600 ± 0.035	0.287 ± 0.024	nd	nd	nd
3	3.00	15.477 ± 0.045	2.727 ± 0.010	0.383 ± 0.016	0.052 ± 0.008	0.077 ± 0.013	1.200 ± 0.010
4	4.00	14.890 ± 0.165	2.620 ± 0.061	0.387 ± 0.045	nd	nd	1.028 ± 0.019
5	4.50	15.318 ± 0.020	2.695 ± 0.014	0.368 ± 0.028	0.050 ± 0.003	0.077 ± 0.006	1.092 ± 0.079
6	5.00	14.984 ± 0.016	2.622 ± 0.067	0.389 ± 0.040	nd	0.106 ± 0.029	1.035 ± 0.036
7	5.50	15.267 ± 0.049	2.655 ± 0.028	0.385 ± 0.050	0.054 ± 0.008	0.072 ± 0.005	1.030 ± 0.002
8	6.00	15.809 ± 0.031	2.808 ± 0.022	0.396 ± 0.006	0.051 ± 0.007	0.081 ± 0.018	1.386 ± 0.010
pooled SD		0.077	0.037	0.032	0.008	0.017	0.037
pooled CV (%)		0.50	1.39	8.87	16.73	20.31	3.24
sample variance (%)		6.4	24.1	39.5	77.8	75.7	6.3
group variance (%)		93.6	75.9	60.5	22.2	24.3	93.7
isotope ratio (theory)		15.025	2.451	0.212	0.014		

[a] nd, not detected.

Table 2. Corrected Enrichment (×100) of Glucose Isotopomers from a Subject Receiving a Constant Infusion of [U-$^{13}C_6$]Glucose

sample	time (h)	[M + 1]	[M + 2]	[M + 3]	[M + 4]	[M + 5]	[M + 6]
1	−0.50	0.185 ± 0.031	0.001 ± 0.014	0.011 ± 0.022	0.016 ± 0.011	−0.004 ± 0.001	0.000 ± 0.000
2	−0.25	−0.122 ± 0.112	0.003 ± 0.018	0.008 ± 0.028	−0.027 ± 0.003	0.001 ± 0.000	0.000 ± 0.000
3	3.00	0.122 ± 0.044	0.093 ± 0.013	0.084 ± 0.017	0.008 ± 0.006	−0.002 ± 0.013	1.164 ± 0.009
4	4.00	−0.458 ± 0.164	0.070 ± 0.042	0.105 ± 0.047	−0.042 ± 0.005	−0.059 ± 0.001	1.004 ± 0.019
5	4.50	−0.036 ± 0.020	0.084 ± 0.013	0.075 ± 0.025	−0.008 ± 0.027	0.007 ± 0.012	1.058 ± 0.077
6	5.00	−0.365 ± 0.016	0.059 ± 0.064	0.106 ± 0.031	−0.042 ± 0.005	0.041 ± 0.028	1.000 ± 0.035
7	5.50	−0.086 ± 0.049	0.052 ± 0.022	0.096 ± 0.046	−0.008 ± 0.036	0.005 ± 0.003	0.998 ± 0.001
8	6.00	0.450 ± 0.031	0.126 ± 0.025	0.085 ± 0.008	0.004 ± 0.007	−0.008 ± 0.017	1.345 ± 0.011

IPAc derivative is less than that of glucose pentaacetate or silylated derivatives, making it attractive for isotopic abundance measurements using GC-combustion-isotope ratio mass spectrometry. Although in this work we used the acetylated derivative to improve capillary GC performance, the final acetylation step could be eliminated to further minimize the exogenous isotope dilution burden.

Using a simple modification, the pentafluorobenzoyl ester derivative can be made. The PFBz derivatives are about 100-fold more sensitive when analyzed by NCI GC/MS than the acetate derivative, making them attractive for studies in infants and other subjects from whom limited amounts of blood are available. In addition, studies of galactose, mannose, and fructose metabolism become practical. These carbohydrates are normally absent in the fasting state, and their concentration in the fed state may be more than 50-fold lower than that of glucose. The NCI spectrum has a dominant molecular anion, unlike many PFBz esters that produce mainly a pentafluorobenzoyl anion. Because glucose is present at about 5−10 mmol L^{-1} in plasma, the EI methods are adequate for most routine isotopomer analyses.

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