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Endogenous Glucose Production in Patients With Glycogen Storage Disease Type Ia Estimated by Oral \(\text{d-}[6,6-{^2}\text{H}_2]\)-glucose

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Abstract

Context: Glycogen storage disease type Ia (GSDIa) is an inborn metabolic disorder characterized by impaired endogenous glucose production (EGP). Monitoring of patients with GSDIa is prioritized because of ongoing treatment developments. Stable isotope tracers may enable reliable EGP monitoring.

Objective: The aim of this study was to prospectively assess the rate of appearance of endogenous glucose into the bloodstream \((R_a)\) in patients with GSDIa after a single oral \(\text{d-}[6,6-{^2}\text{H}_2]\)-glucose dose.

Methods: Ten adult patients with GSDIa and 10 age-, sex-, and body mass index–matched healthy volunteers (HVs) were enrolled. For each participant, 3 oral glucose tracer tests were performed: (1) preprandial/fasted, (2) postprandial, and (3) randomly fed states. Dried blood spots were collected before \(\text{d-}[6,6-{^2}\text{H}_2]\)-glucose administration and 10, 20, 30, 40, 50, 60, 75, 90, and 120 minutes thereafter.

Results: Glucose \(R_a\) in fasted HVs was consistent with previously reported data. The time-averaged glucose \(R_a\) was significantly higher in (1) preprandial/fasted patients with GSDIa than HV and (2) postprandial HV compared with fasted HV \((P<.05)\). A progressive decrease in glucose \(R_a\) was observed in preprandial/fasted patients with GSDIa; the change in glucose \(R_a\) time-course was directly correlated with the change in capillary glucose \((P<.05)\).

Conclusion: This is the first study to quantify glucose \(R_a\) in patients with GSDIa using oral \(\text{d-}[6,6-{^2}\text{H}_2]\) glucose. The test can reliably estimate EGP under conditions in which fasting tolerance is unaffected but does not discriminate between relative contributions of EGP (eg, liver, kidney) and exogenous sources (eg, dietary cornstarch). Future application is warranted for longitudinal monitoring after novel genome based treatments in patients with GSDIa in whom nocturnal dietary management can be discontinued.

Key Words: glycogen storage disease type Ia, stable isotopes, diet, monitoring, precision medicine

Abbreviations: BMI, body mass index; CBG, capillary blood glucose; CGM, continuous glucose monitoring; CNGDF, continuous nocturnal gastric drip feeding; DBS, dried blood spot; EGP, endogenous glucose production; GCMS, gas chromatography mass spectrometry; GSDIa, glycogen storage disease type Ia; HV, healthy volunteer; \(R_a\), rate of appearance; SIB, stable isotope blood; UCCS, uncooked corn starch.

Glycogen storage disease type Ia (GSDIa) is an inborn disorder of carbohydrate metabolism characterized by severe fasting hypoglycemia due to impaired endogenous glucose production (EGP). GSDIa is caused by glucose 6-phosphatase-a (G6Pase-a) deficiency due to biallelic G6PC1 gene variants, resulting in defective glycogenolysis and gluconeogenesis, which in turn impairs EGP. Strict medically prescribed nutrition therapy is the cornerstone of the treatment (1). Although controversies still exist (2), the medically prescribed dietary regimens include frequent daytime feedings which are restricted in fructose, sucrose and (g)alactose, as a form of substrate reduction. In addition, uncooked cornstarch (UCCS), extended-release cornstarch (Glycosade) or continuous nocturnal gastric drip-feeding (CNGDF) serve as product supplementation (3, 4). The general aims of these strict diets is to achieve normoglycemia, to normalize secondary metabolic perturbations, and to prevent long-term, chronic complications as much as possible. Liver transplantation is an increasingly recognized alternative treatment option; its advantages and disadvantages need to be weighed individually (5).
Novel treatment approaches for GSDIa are currently being investigated, including adeno-associated virus (AAV) serotype 8 (AAV8)-mediated gene therapy (NCT05139316) and mRNA-mediated therapy (NCT05095727). In vivo genome editing is in clinical development following promising results in GSDIa mice (6). Ideally, because of these novel treatments, EGP would be restored, allowing patients with GSDIa no longer being dependent on strict medically prescribed diets and safely sleep throughout the night, without hypoglycemia risks.

The recent multistakeholder international priority setting partnership for liver GSDs has emphasized the need for new, low burdensome methods for monitoring metabolic control (7). Appropriate monitoring of patients with GSDIa is crucial to reliably assess the acute and long-term efficacy of any treatment. Among the primary outcome parameters used in the aforementioned clinical trials are changes in (1) the percentage time-in-range as measured by continuous glucose monitoring (CGM) (8), and (2) time to hypoglycemia assessed by in-hospital controlled fasting challenges.

In theory, stable isotope methods have the potential to monitor EGP in patients with GSDIa in a reliable, longitudinal, minimally invasive, safe fashion (9). At least 2 major methodological aspects challenge the use of stable isotopes in patients with GSDIa. First, most approaches require intravenous or nasogastric tube administration of stable isotope tracers using a primed continuous infusion and repeated venous blood sampling during the test. Second, patients with GSDIa depend on dietary treatment to maintain euglycemia and cannot be studied safely under fasted conditions. Consequently, any stable isotope approach is likely to result in EGP overestimation due to the contribution of unlabeled exogenous dietary glucose, which increases the total glucose rate of appearance ($R_a$; ie, the rate of endogenous glucose production by liver, kidney and intestine, plus any glucose originating from dietary sources).

In recent years, intraperitoneal or oral stable isotope-labeled glucose administration followed by serial dried blood spot (DBS) sampling has proven a valid approach to assess glucose $R_a$ and EGP in small laboratory animals (10-13). Since these animals were fasted, glucose $R_a$ was equal to EGP (10-13). These studies suggest that oral administration of glucose tracers may provide a minimally invasive approach to estimate glucose turnover in humans. The aim of the current study was to overcome the limitations of traditional stable isotope approaches by developing a minimally invasive method enabling $R_a$ quantification in patients with GSDIa which can be translated in regular care and clinical studies. Here we report our prospective, investigator-initiated human pilot study in 10 adult patients with GSDIa and 10 age-, sex-, and body mass index (BMI)-matched healthy volunteers (HVs) receiving a single oral $\text{}[\text{6,6}-\text{H}_2]\text{-glucose dose. For each participant, glucose } R_a \text{ was estimated under 3 conditions: overnight fasted before breakfast, postprandially after lunch, and at a random time.}$

### Subjects and Methods

#### Study Approval

The Medical Ethics Committee of the University Medical Center Groningen (UMCG), The Netherlands approved the study protocol (ref. no. METc 2020/342). The study was conducted according to the principles of the Helsinki Declaration of 1975 as revised in 2013. All participants provided written informed consent prior to inclusion in the study.

#### Study Design

This was a prospective, investigator-initiated human pilot study (ENGILUPRO GSDIa, NCT04311307). Study days 1 and 2 were scheduled at the UMCG after written informed consent was signed by the participants. Information on participants’ demographic, genotype (for patients with GSDIa), and diet was collected. The procedures on study days 1 and 2 were performed under the supervision of a research nurse and a physician. The procedures on study day 3 were performed at home (or in hotel, in case of participants’ preferences) by the participants without supervision, after participants were provided with detailed instructions and written information during study days 1 and 2. Participants stayed at the hotel (or at home, in case of participants’ preferences) during the night between day 1 and 2. All participants were asked to return the study material to the study site after completion of all study procedures.

#### Study Participants

The trial was conducted at the UMCG between October 2020 and July 2021. Patients with GSDIa and an equal number of age-, gender- and BMI–matched HVs were recruited at the UMCG. Inclusion criteria were (1) age >16 years, (2) stable medical condition before the start of the test procedures and for patients with GSDIa, and (3) confirmation of GSDIa with enzyme assay and/or $G6PC1$ variation analysis. Exclusion criteria included (1) pregnancy, (2) recent (<1 month) history of hospitalization due to hypoglycemia, (3) intercurrent illness (defined as a combination of decreased dietary intake, vomiting, diarrhea, and fever (>38.5 °C) in the week prior to the study visit), and for HVs also (4) confirmed diagnosis or history suggestive of diabetes mellitus, (5) first-grade family member with a confirmed diagnosis associated with fasting intolerance, and (6) symptoms or signs by suggestive of fasting intolerance, metabolic instability, fever or gastrointestinal complaints.

Based on their $G6PC1$ genotype (or clinical features in case the genotype was not available), patients with GSDIa were classified as “severe” (2 nonsense or active site variants or clinical ascertainment before the age of 2 years) or “attenuated” (2 missense variants not in the active site or 1 nonsense or active site variant and 1 missense variant not in the active site or clinical ascertainment after the age of 2 years). Variants were reported according to ClinVar, or based on published literature in case a mutation was not published/deposited on ClinVar. Each participant was assigned a code which was used to label the study materials (ie, data collection forms, biological material, cloud data).

#### Study Procedures

The study protocol is presented in Fig. 1. For each participant, 3 glucose stable isotope blood load tests (referred to as “glucose-SIB tests”) were performed. The first 2 glucose-SIB tests were performed at the study site under supervised conditions, during which 1.25 g of $\text{}[\text{6,6}-\text{H}_2]\text{-glucose dissolved in water (referred to as the “study drink”) were either orally administered before breakfast (glucose-SIB test 1) or after lunch (glucose-SIB test 2). The glucose-SIB test 3 was performed in
an outpatient setting (at home or hotel) without supervision at a random time, after careful instruction of the study participant.

For each glucose-SIB test, data were collected at multiple time points, namely at baseline just before taking the study drink, and subsequently at 10, 20, 30, 40, 50, 60, 75, 90, 120 (and 180 if available) minutes after taking the study drink. For each time point the following data were collected: 2 DBS samples, capillary blood glucose (CBG) concentrations (capillary blood was obtained by fingerprick), CGM values, and information on any symptoms or signs. The glucose-SIB test was stopped in case (1) the CBG dropped to <3.6 mmol/L and the participant showed other signs or symptoms of hypoglycemia or (2) the participant requested to discontinue the experiment.

On day 1, a CGM device was placed (Dexcom G6) in either the upper arm or the abdomen. Instructions on how to appropriately use the CGM device were provided by an experienced research nurse. Additionally, all participants (patients and volunteers) were given the same type of CBG device (Freestyle freedom Lite) to be used for the entire study. Participants were also instructed on how to fill a food diary during the entire study.

On day 2, glucose-SIB test 1 was started between 05:00 and 06:00 AM and before breakfast, according to each participant’s feeding pattern. For patients with GSDIa, the timing of the last meal prior to glucose-SIB test 1 was accommodated to their usual fasting tolerance. HVs were asked to fast for at least 8 hours. For 3 patients with GSDIa, proper fasting was not possible due to CNGDF. In these patients, glucose-SIB test 1 was performed during the last 2 hours of CNGDF. Glucose-SIB test 2 was started between 12:00 and 02:00 PM on day 2, after taking lunch according to each participant’s regular (prescribed) nutrition regimen. For glucose-SIB test 1 and 2, detailed information on the last (or concurrent) meal was recorded for each participant. On day 2 all participants were provided with instructions for glucose-SIB test 3, CGM, and shipping of the study material back to the site.

In order to minimize the risk of CGM malfunction and/or disconnection during glucose-SIB test 3, participants were asked to perform this test between day 3 and 7 and to send the study material back to the site on study day 10.

Noninvestigational Medical Product: The “Study Drink”

The D-[6,6-2H2]-glucose was purchased from Cambridge Isotope Laboratories, Inc. (Cambridge MA, USA). The study drink was prepared by Apotheek A15, and purity was confirmed to be 97%. The study drink was stored at the UMCG pharmacy at the study site according to good manufacturing practice. For each participant, 3 vials (ie, 1 vial for each glucose-SIB test) were distributed to the investigators 1 day before study day 1. Each study vial contained 1.25 g of D-[6,6-2H2]-glucose powder (each glucose molecule carried 2 hydrogen atoms as deuterium with a molar mass of 182.17) and was labeled with the participant’s study code. This dose was based on pilot studies previously performed in healthy subjects (T. H. van Dijk, unpublished observations). The D-[6,6-2H2]-glucose powder was dissolved in water in 2 steps (total volume of 200 mL) by the investigators for glucose-SIB tests 1 and 2, and by the participants for glucose-SIB test 3 after instruction.

Outcome Measures

The primary study endpoint was assessing glucose $R_g$. Calculated glucose $R_g$ was compared between (1) patients with GSDIa vs matched HVs; (2) severe and attenuated patients with GSDIa; (3) the preprandial and the fed state (ie, glucose-SIB test 1 and glucose-SIB test 2) in subjects with GSDIa; (4) the preprandial and fed state (ie, glucose-SIB test

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**Figure 1.** Study protocol. T1, glucose-SIB test 1 (before breakfast); T2, glucose-SIB test 2 (after lunch); T3, glucose-SIB test 3 (random time); *supervised; #unsupervised; +1 additional sample at +180 was collected in a subset of participants. Light grey arrows, start; black arrows, end.
1 and glucose-SIB test 2) in HVs; (5) the controlled hospital setting and at home setting (ie, glucose-SIB test 1 and glucose-SIB test 3) in patients with GSDIa; (6) the controlled hospital setting vs at home setting (ie, glucose-SIB test 1 and glucose-SIB test 3) in HVs. CBG and CGM data were collected and stored as exploratory endpoints and these results have been published previously (14).

### Analytical Procedures

#### Glucose derivatization and gas chromatography mass spectrometry measurements

Collected DBSs were air dried horizontally for at least 3 hours at room temperature avoiding direct sunlight. Sample preparation for gas chromatography mass spectrometry (GCMS) analysis to determine the fractional distribution of glucose isotopomers was performed according to Van Dijk et al (15). Briefly, a 6.5-mm disk was punched out from each DBS, wetted with 50 μL of water for 15 minutes followed by the addition of 500 μL of ethanol, and incubated overnight at room temperature. After centrifuging, 400 μL of the supernatant was transferred to a Teflon-capped reaction vial and dried at 60 °C under a stream of N₂. Glucose was subsequently converted to its pentaacetate derivative by adding 300 μL of pyridine/acetic anhydride (1:2) to the residue and incubating for 30 minutes at 60 °C (or overnight at room temperature). After drying at 60 °C under a stream of N₂, the residue was dissolved in 200 μL of ethylacetate and transferred into an injection vial for GCMS analysis ([GC, Agilent 7890A; MS, Agilent 5975C in MS Selective Detector (MSD)]; Agilent Technologies, Amstelveen, The Netherlands). Derivatives were separated on a Zebron ZB-1701 30 m x 0.25 mm ID (0.25 μM film thickness) capillary column (Phenomenex, Utrecht, The Netherlands). Mass spectrometric analyses were performed by positive chemical ionization with ammonia. Out of the ions monitored, namely, m/z 408 to 412 (m0-m4), the fractional contribution of n-[6,6-²H₂]-glucose in blood glucose is represented by M2, which was used for calculations.

#### Correction for naturally occurring isotopes and assessment of isotopomer data quality

The isotopologue distributions obtained by GCMS analysis were corrected for naturally occurring isotopes as previously described (15). The correction was based on the measured average isotopologue distribution in the baseline samples of all glucose-SIB test (ie, samples collected just before the study drink was taken). In most baseline samples the measured M2 fraction deviated less than 0.3% from the theoretical prediction by ChemCalc (https://www.chemcalc.org/) (16). Only baseline samples from participant 004_glucose-SIB test 3, participant 008_glucose-SIB test 2, and participant 018_glucose-SIB test 2 contained 0.45, 0.96, and 0.39% excess M2 compared with the theoretical prediction, respectively. To account for the possibility that certain foods consumed by these participants affected the basal isotopomer abundances (17), the data from these 3 tests were corrected for the participant’s own baseline sample of the respective glucose-SIB test.

#### Model description

To derive the Rₐ from the tracer data, we used the 2-compartment model and parameter fitting procedure recently described by Vieira-Lara et al (13). The difference with the latter study design was that our subjects received only ²H₂-labeled glucose in the study drink, whereas they supplied a mixture of labeled and unlabeled glucose. This was taken into account in the modeling of the data.

In brief, we considered 2 compartments, the external (stomach) compartment (compartment 1) and the blood plasma (compartment 2). The pool sizes (q) and concentrations (c) of labeled glucose were denoted by q₁ and q₂, c₁, and c₂ respectively, and those of unlabeled glucose in plasma analogously by Q₂ and C. Pool sizes Q and q were expressed in mmol/kg (referring to kg body weight), and concentrations C and c in mmol/L.

The tracer model consisted of 3 rates ν (Fig. 2): import of glucose into the blood plasma compartment (ν₁), elimination from the plasma into the tissues (ν₂); and metabolism or storage of glucose before it enters into the blood (ν₃, with subscript L denoting loss). All rates ν were expressed in mmol/kg/minute and described by first-order kinetics, with rate constants k per minute with the following rate equations for the tracer:

\[ ν₁(q₁) = k₁ · q₁ \]
\[ ν₃(c₂) = k₂ · c₂ · Vol \]

The conversion factor Vol in mL kg⁻¹ was the distribution volume of the plasma compartment and served as a conversion factor between concentration and pool size. Analogously, the elimination rate of the unlabeled glucose was defined by:

\[ ν₂(c₂) = k₂ · c₂ · Vol \]
\( v_2(C_2) = k_2 \cdot C_2 \cdot \text{Vol} \)

In contrast to the model of Vieira-Lara (13), current model lacked the equations for uptake and loss of unlabeled glucose since the bolus did not contain unlabeled glucose in the present study. Finally, the rate of appearance of unlabeled glucose was quantified as the \( R_a^{-} \) in mM/minute or \( R_a^{+} \) in µmol/kg/minute. This then led to the following set of Ordinary Differential Equations (ODEs):

\[
\begin{align*}
\frac{dq_1}{dt} &= -(k_1 + k_L) \cdot q_1 \\
\frac{dc_2}{dt} &= \frac{k_1 \cdot q_1}{\text{Vol}} - k_2 \cdot c_2 \\
\frac{dC_2}{dt} &= -k_2 \cdot C_2 + R_a^{*}(t)
\end{align*}
\]

By implication time \( t \) was expressed in minutes.

**Calculation of \( R_a \) from the tracer time courses**

The analytical solution of the above-described model as derived before (13) reads:

\( c_2(t) = C \cdot (e^{-k_2 \cdot t} - e^{-k_L \cdot t}) \)

with: \( k_2 = k_1 + k_L \)

The \( c_2 \) time courses were obtained by multiplication of the measured total glucose concentrations and the measured \( M_2 \) enrichment at each time point. Based on the data of all subjects and tests in this study, and following the procedure outlined by Vieira-Lara (13), \( C \) was estimated from the data at 0.34 mM and \( k_2 \) was constrained between 0.028 and 0.33 minute\(^{-1}\). Subsequently, \( k_2 \) and \( k_2 \) were fitted to each individual tracer time course, yielding their values for each individual test subject and test. It can be derived (13) that:

\[ C = \frac{q_1(0) \cdot F}{\text{Vol}} \cdot \frac{k_2}{k_2 - k_L} \]

in which \( q_1(0) \) is the amount of tracer administered and \( F \) (dimensionless) the bioavailability of the tracer (ie, the fraction of the tracer that reaches the sampled plasma pool):

\[ F = \frac{k_1}{k_1 - k_L} \]

We assumed a constant bioavailability \( F \) (equal for all subjects) of 0.89 based on triple-tracer tests with 88 healthy subjects (18). This then allowed the distribution volume \( \text{Vol} \) to be computed:

\[ \text{Vol} = \frac{q_1(0) \cdot F}{C} \cdot \frac{k_2}{k_2 - k_L} \]

We note that an independent assessment of \( \text{Vol} \) and \( F \) would have required intravenous (IV) administration of the label.

First, the time-averaged \( R_a \) was computed, based on the fitted \( k_2 \). To this end, the time-averaged tracee concentration \( (C_{2,\text{avg}}) \) was computed by integrating the concentration of unlabeled glucose over time (area under the curve) and dividing by the duration of the test \( (t_{\text{end}} - t_0) \). A steady state was assumed for the tracee concentration. At steady state the rate of glucose elimination from the plasma pool equals the rate of appearance, and thus:

\[ R_a^{*,\text{average}} = k_2 \cdot C_{2,\text{avg}} \cdot \text{Vol} \]

Second, only for glucose-SIB test 1 (ie, preprandial/fastened conditions) the \( R_a \) was computed as a function of time. The time course of unlabeled glucose was fitted to a linear function:

\[ C_2(t) = a + b \cdot t \]

Hence:

\[ \frac{dC_2(t)}{dt} = b \]

At the same time (above):

\[ \frac{dC_2(t)}{dt} = -k_2 \cdot C_2(t) + R_a^{*} \]

Setting the 2 expressions equal to each other gives:

\[ R_a^{*}(t) = b + k_2 \cdot C_2(t) \]

To convert \( R_a^{*} \) in mM to \( R_a \) in µmol/kg/minute, \( R_a^{*} \) was multiplied by the distribution volume \( \text{Vol} \). Note that the estimated \( R_a \) in µmol/kg/minute is based on an assumed bioavailability \( F \) of 0.89 (18) whereas the estimated \( R_a^{*} \) in mM is independent of \( F \).

In 3 tests, \( k_2 \) could not be calculated, since there was a less than 25% decrease between the peak concentration of \( \nu \{[6,6-\text{H}_2]\}-\text{glucose} \) and the concentration at the last time point. This concerned participant 008 (HV) glucose-SIB test 1 (25% decrease), participant 019 (HV) glucose-SIB test 2 (2% decrease), and participant 020 (GSDIa attenuated) glucose-SIB test 2 (6% decrease). These tests were excluded from further computational analysis (file 1 (19)).

**Software and Statistical Analysis**

Correction for natural abundance of isotopes was performed in Excel 2019. All other calculations on glucose kinetics were done in Python, using Jupyter Notebook 6.1.4. Statistical analysis was performed using IBM SPSS Statistics 23. The comparisons between numerical variables were performed by Student’s t-test corrected for Fisher’s exact test. The normality of the distribution was checked by the Shapiro-Wilk test. Comparison between CBG curves generated in patients with GSDIa and HVs both during glucose-SIB test 1 and glucose-SIB test 2 was performed by 2-way analysis of variance analysis with the Geisser–Greenhouse correction. CBG and CGM values collected during glucose-SIB test 3 were excluded from the analysis of variance analysis in order to minimize possible bias due to data collected under unsupervised conditions. Correlation study was performed by Spearman’s rank correlation. Statistical significance was set at \( P < .05 \). For results on (1) CBG values, (2) comparison between CBG and CGM, and (3) correlation between change in glucose \( R_a \) and change in CBG, patients with GSDIa were analyzed as a group \( (n = 10) \). For results on (1) glucose \( R_a \) (both time-averaged and time courses) and (2) \( M_1 \) fraction patients with GSDIa with an attenuated \( (n = 6) \) and a severe \( (n = 4) \) phenotype were analyzed separately, unless stated otherwise.

**Results**

**Study Participants**

Ten patients with GSDIa (5 females, 5 males) with a median age of 22.2 years (range 17.8–53.1) and a median BMI of 26.1 kg/m² (range 22.4–29.8) were enrolled. Ten age-,
gender-, and BMI-matched HVs were also enrolled. Table 1 presents the clinical and genetic characterization of the patients with GSDIa, and the details of their nutrition diary are summarized in Table 2.

Safety
No serious adverse events were recorded during the glucose-SIB tests. Three patients with GSDIa showed asymptomatic CBG <3.6 mmol/L between 90 and 120 minutes during glucose-SIB test 1. These events were deemed unrelated to the study drink and most likely related to GSDIa.

Glucose Concentrations
CBG data collected under supervised conditions (ie, glucose-SIB tests 1 and 2) are presented in Fig. 3 and elsewhere (file 2 (19)). During glucose-SIB test 1 (ie, supervised preprandial/fasted condition), no major increases in glucose concentrations were observed after ingestion of the study drink in any of the participants. As expected, based on the metabolic defect, a significant progressive decrease in glucose concentrations during glucose-SIB test 1 was observed in patients with GSDIa compared with HVs (P < .01) (Fig. 3A and 3B). A major increase in glucose concentrations was observed during glucose-SIB test 2 (supervised postprandial condition) in both patients with GSDIa and HVs. No difference between the groups was observed (P > .05) (Fig. 3C and 3D). A comparison of the CBG and CGM measurements was previously reported elsewhere (14).

Glucose R2
Fifty-nine M2 glucose curves (glucose-SIB test 1, n = 20 [10 GSDIa and 10 HVs]; glucose-SIB test 2, n = 20 [10 GSDIa and 10 HVs]; glucose-SIB test 3, n = 19 [10 GSDIa and 9 HVs]) were generated. For participant 012, CBG was not collected during glucose-SIB test 3 and therefore an M2 curve could not be generated. Data from the 56 approved tests were subsequently used for computational modeling. Kinetic constants and other model parameters are presented elsewhere (file 3 (19)). Time-averaged glucose R2 and glucose R2 time courses are presented in Table 3 and Fig. 4. The time-averaged glucose R2 was significantly higher in patients with GSDIa than in HVs (P < .05) (for both R2 in µmol/kg/minute and mM/minute) in glucose-SIB test 1 (ie, supervised preprandial/fasted condition) (Fig. 4A). No significant differences in the time-averaged glucose R2 between patients with GSDIa and HVs were observed in glucose-SIB test 2 (ie, supervised postprandial condition) and glucose-SIB test 3 (ie, unsupervised random fed condition) for both glucose R2 in µmol/kg/minute and mM/minute (Fig. 4B and 4C). Mean glucose R2 in HV was significantly higher in glucose-SIB test 2 than glucose-SIB test 1 (P < .05) for both R2 in µmol/kg/minute and mM/minute but did not differ significantly between glucose-SIB tests 1 and 2 in patients with GSDIa (Fig. 4A and 4B). No significant correlation between the time-averaged glucose R2 and (1) fasting time before glucose-SIB test 1, (2) carbohydrate intake before glucose-SIB test 2, (3) daily UCCS/Glycosade intake, or (4) overnight UCCS/Glycosade intake was found in patients with GSDIa. Time courses of glucose R2 during glucose-SIB test 1 showed a stable trend in HVs and a progressive decrease in patients with GSDIa (Fig. 4D). The median decrease in glucose R2 during glucose-SIB test 1 was 28.5% in patients with GSDIa with an attenuated phenotype and 14% in patients with GSDIa with a severe phenotype. No significant correlation between glucose R2 calculated either at baseline or at the end (+120 minutes) of glucose-SIB test 1 and fasting time prior to glucose-SIB test 1 was found in patients with GSDIa. Among patients with GSDIa, the change in glucose R2 (ie, the difference between the glucose R2 at the end of the test and the glucose R2 at baseline) was directly correlated with the change in CBG (ie, the difference between the CBG concentration at the end of the test and the CBG at baseline) during glucose-SIB test 1 (Fig. 5).

Discussion
This is the first study in patients with GSDIa aiming to quantify glucose R2 using a single oral d-[6,6-2H2]-glucose dose. We demonstrated that oral administration of d-[6,6-2H2]-glucose combined with DBS sampling allows estimation of glucose R2 both in patients with GSDIa and HVs. Moreover, we showed that calculated glucose R2 is influenced by the subject feeding status (eg, frequent feedings and UCCS/Glycosade). As such, glucose R2 likely exceeded EGP in patients with GSDIa and fed HVs due to the contribution of unlabeled dietary glucose. Assessing EGP in patients with GSDIa has been a key research challenge for decades. In theory, EGP represents an ideal biomarker for monitoring patients with GSDIa. Firstly, it is directly influenced by defective G6Pase-α activity. Secondly, it is significantly lower in patients with GSDIa than in the healthy population. Thirdly, it can be monitored over time. EGP can be estimated by (stable isotope labeled) tracer studies (20). Hence, proper background knowledge on physiology and stable isotope technique is essential to adequately interpret the data. EGP represents the amount of glucose that is physiologically produced by the body to avoid hypoglycemia. The liver ensures up to ~80% of EGP, with the remainder largely accounted for by the kidneys (21). Biochemically, 2 main processes contribute to EGP, namely gluconeogenesis and glycogenolysis (22). When stable isotope tracers are employed, the glucose R2 refers to the sum of unlabeled glucose produced endogenously by the organs (ie, EGP), plus that from exogenous sources (eg, diet, IV glucose) appearing per unit of time in the bloodstream (23).

Intriguingly, previous stable isotope studies have shown that patients with GSDIa display considerable residual EGP despite (often almost complete) G6Pase-α deficiency (Table 4 (24-31)). In those studies, EGP was derived from the glucose R2. Since many adult patients with GSDIa require frequent meals and UCCS every 4 to 6 hours (or alternatively IV glucose infusion or carbohydrates administration via nasogastric tube) to maintain euglycemia, the EGP was likely overestimated. This limitation applies to any approach aiming at quantifying EGP in patients with GSDIa. Importantly, different tracers, administration routes and feeding conditions were used in previous studies, potentially contributing to the variability observed among patients with GSDIa (24, 31-33).

Based on previous encouraging results obtained in non-GSDIa mice (10, 15) and taking into account the above-mentioned considerations, we tested the feasibility of glucose R2 and EGP quantification in adult patients with GSDIa after a single oral d-[6,6-2H2]-glucose dose. Collected data showed that this method allows to quantify glucose R2 in adult patients with GSDIa and HVs. It is unlikely, however, that the
<table>
<thead>
<tr>
<th>Participant</th>
<th>Age (years)</th>
<th>Gender</th>
<th>BMI (kg/m²)</th>
<th>Genotype (G6PC1 variants)</th>
<th>Clinical ascertainment years</th>
<th>Phenotype</th>
<th>Dietary management</th>
<th>Nocturnal carbohydrate intake (g/kg/hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>001</td>
<td>44.1</td>
<td>F</td>
<td>25.3</td>
<td>c.809G&gt;T, c.1039C&gt;T</td>
<td>p.Gly270Val, p.Gln347Ter</td>
<td>&lt;0.1 (diagnosed before symptoms onset following her elder sibling)</td>
<td>attenuated</td>
<td>Frequent feedings Glycosade (3.2 g/kg/day)</td>
</tr>
<tr>
<td>002</td>
<td>21.6</td>
<td>M</td>
<td>29.8</td>
<td>c.189G&gt;A, c.189G&gt;A</td>
<td>p.Trp63Ter, p.Trp63Ter</td>
<td>0.7</td>
<td>Severe</td>
<td>Frequent feedings Glycosade (2.2 g/kg/day)</td>
</tr>
<tr>
<td>004</td>
<td>17.8</td>
<td>F</td>
<td>22.4</td>
<td>c.1039C&gt;T, c.1039C&gt;T</td>
<td>p.Gln347Ter, p.Gln347Ter</td>
<td>1.8</td>
<td>Severe</td>
<td>Frequent feedings Glycosade (1.6 g/kg/day)</td>
</tr>
<tr>
<td>006</td>
<td>53.1</td>
<td>F</td>
<td>27.3</td>
<td>c.1039C&gt;T, c.247C&gt;T</td>
<td>p.Gln347Ter, p.Arg83Cys</td>
<td>N.A.</td>
<td>Severe</td>
<td>Frequent feedings Glycosade (2.4 g/kg/day)</td>
</tr>
<tr>
<td>007</td>
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<td>M</td>
<td>29.5</td>
<td>c.1039C&gt;T, c.247C&gt;T</td>
<td>p.Gln347Ter, p.Arg83Cys</td>
<td>3.5</td>
<td>Severe</td>
<td>Frequent feedings Glycosade (2.1 g/kg/day) CNGDF</td>
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<td>F</td>
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<td>Attenuated</td>
<td>Frequent feedings CNGDF</td>
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<td>014</td>
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<td>F</td>
<td>25.6</td>
<td>c.247C&gt;T, c.187T&gt;C</td>
<td>p.Arg83Cys, p.Trp63Arg</td>
<td>0.5</td>
<td>Attenuated</td>
<td>Frequent feedings Glycosade (3.2 g/kg/day)</td>
</tr>
<tr>
<td>015</td>
<td>19.3</td>
<td>M</td>
<td>23.0</td>
<td>c.247C&gt;T, c.187T&gt;C</td>
<td>p.Arg83Cys, p.Trp63Arg</td>
<td>&lt;0.1 (diagnosed before symptoms onset following his elder sibling)</td>
<td>Attenuated</td>
<td>Frequent feedings Glycosade (3.1 g/kg/day)</td>
</tr>
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<td>017</td>
<td>18.3</td>
<td>M</td>
<td>26.6</td>
<td>c.247C&gt;T, c.866G&gt;A</td>
<td>p.Arg83Cys, p.Ser289Asn</td>
<td>N.A.</td>
<td>Attenuated</td>
<td>Frequent feedings UCCS (1.9 g/kg/day) CNGDF</td>
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<td>48.3</td>
<td>M</td>
<td>26.9</td>
<td>c.809G&gt;T, c.1039C&gt;T</td>
<td>p.Gly270Val, p.Gln347Ter</td>
<td>0.4</td>
<td>Attenuated</td>
<td>Frequent feedings UCCS + Glycosade (2.3 g/kg/day)</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>22.4</td>
<td>5 M</td>
<td>23.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>(17.1-50.8)</td>
<td>5 F</td>
<td>(19.8-28.9)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; CNGDF, continuous nocturnal gastric drip-feeding; N.A., not available; UCCS, uncooked cornstarch.

*Median and range are shown.*
estimated $R_a$ exclusively represents EGP in patients with GSDIa. The contribution of UCCS may explain why the glucose $R_a$ in test 1 was higher in patients than in HVs, since the HVs had fasted for at least 8 hours.

Time-averaged glucose $R_a$ in preprandial patients was 7.7 to 27.0 µmol/kg/minute (1.4-4.9 mg/kg/minute). Our results are in line with previous studies (Table 4), confirming the reported variability among patients with GSDIa. In this study that included a larger number of patients with GSDIa, glucose $R_a$ was significantly higher in preprandial patients with GSDIa compared to HVs while no major differences were observed between patients and HVs in (random) fed states. Although no correlation between glucose $R_a$ and fasting time prior to glucose-SIB test 1 and carbohydrate intake prior to glucose-SIB test 2 or daily UCCS intake was found in patients with GSDIa, results support the role of exogenous sources (ie, diet) to the estimated glucose $R_a$ in patients. The slowly digested UCCS likely represented a major contributor to the estimated UCCS likely represented a major contributor to the estimated glucose $R_a$ in patients with GSDIa. Cornstarch is relatively highly enriched in $^{13}$C (17, 34). Yet, the baseline $M_1$ fraction in blood glucose observed in patients with GSDIa (0-0.2%) was found to be negligible, suggesting that the UCCS used by the patients in this study was not overenriched in $^{13}$C compared with the diet of HVs.

Insulin resistance is observed in GSDIa (35) and UCCS requirements decrease in aging patients with GSDIa (36). Although none of the patients with GSDIa included in this study displayed increased HbA1c levels during regular care visits, these potential contributing factors were not formally assessed in the present study and may have affected glucose $R_a$ in a postprandial state (37).

Time-averaged glucose $R_a$ was 7.5 to 13.9 µmol/kg/minute (1.4-2.5 mg/kg/minute) in fasted HVs. After a physiological overnight fast, glucose metabolism is in steady state, meaning that the rate of glucose entering the bloodstream ($R_s$) equals the flow rate of glucose leaving the bloodstream (rate of disappearance; $R_a$). In this situation there is no contribution of exogenous sources to the glucose $R_a$, such as it occurs in healthy who do not require UCCS. Therefore, it can be assumed that the estimated glucose $R_a$ in fasted HVs is in line with data on EGP previously published upon continuous glucose tracer infusion, supporting the reliability of our method (20). The time course analysis performed on the preprandial/fasted tests revealed a stable glucose $R_a$ in HVs while a progressive decline was observed in patients with GSDIa. The degree of glucose $R_a$ decline directly correlated with the decline in CBG. Moreover, the decline in glucose $R_a$ was sharper in patients with an attenuated phenotype than in those with a severe phenotype. Again, the progressive depletion of the UCCS may have contributed to this finding. The different interval between subsequent UCCS doses (likely broader in patients with GSDIa with an attenuated phenotype than in severely affected patients) may contribute to the difference observed between the 2 patient subgroups. Indeed, mean fasting time prior to glucose-SIB test 1 was shorter in patients with a severe vs attenuated GSDIa phenotypes (2 hours and 24 minutes vs 2 hours and 34 minutes). Whether this difference was statistically significant could not be ascertained in this relatively small study.

For data analysis, conversion of the $R_a$ from mM/minute to µmol/kg/minute required an estimation of the distribution volume of the tracer. To this end it was assumed that the bioavailability of the tracer was equal for all study participants. This did not affect the differences observed between the study groups (either when expressing $R_a$ in mM/minute or µmol/kg/minute). However a potential contribution of residual intestinal and/or hepatic G6Pase activity to the labeled glucose bioavailability cannot be ruled out. Interpatient differences in residual G6Pase activities may affect intestinal uptake and/or hepatic retention, resulting in differences in glucose tracer bioavailability, hence contributing to the observed variability among patients with GSDIa (38, 39). The relatively low number of patients included in the present study reflects the challenge of patient recruitment when studying rare disorders. It is unknown whether the current sample size adequately reflects the large clinical and biochemical heterogeneity observed in GSDIa (40). Despite such limitations in patients with GSDIa, our test can be used to adequately estimate EGP in fasted HVs and presumably under conditions in which fasting tolerance is not impaired, such as diabetes and chronic kidney disease. Theoretically, it may develop into a baseline assessment

### Table 2. Participants’ nutritional details during each of the 3 glucose-SIB tests

<table>
<thead>
<tr>
<th>Patient with GSDIa</th>
<th>Fasting interval before glucose-SIB test 1 (hours)</th>
<th>CH intake before glucose-SIB test 2 (g/kg)</th>
<th>Feeding status during glucose-SIB test 3</th>
<th>Matched HV</th>
<th>Fasting interval before glucose-SIB test 1 (hours)</th>
<th>CH intake before glucose-SIB test 2 (g/kg)</th>
<th>Feeding status during glucose-SIB test 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>001</td>
<td>5</td>
<td>0.8</td>
<td>Fed</td>
<td>003</td>
<td>9</td>
<td>0.8</td>
<td>Fasted</td>
</tr>
<tr>
<td>002</td>
<td>2</td>
<td>0.4</td>
<td>Fed</td>
<td>008</td>
<td>10</td>
<td>0.4</td>
<td>Fed</td>
</tr>
<tr>
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<td>6</td>
<td>0.8</td>
<td>Fed</td>
<td>010</td>
<td>9</td>
<td>0.3</td>
<td>Fasted</td>
</tr>
<tr>
<td>006</td>
<td>1.5</td>
<td>0.5</td>
<td>Fasted</td>
<td>005</td>
<td>10</td>
<td>0.5</td>
<td>Fasted</td>
</tr>
<tr>
<td>007</td>
<td>0.4</td>
<td>1.4</td>
<td>Fed</td>
<td>012</td>
<td>9.5</td>
<td>0.5</td>
<td>Fasted</td>
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<tr>
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<td>011</td>
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<td>014</td>
<td>3</td>
<td>0.6</td>
<td>Fed</td>
<td>013</td>
<td>8</td>
<td>0.6</td>
<td>Fasted</td>
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<tr>
<td>015</td>
<td>3.5</td>
<td>0.6</td>
<td>Fed</td>
<td>016</td>
<td>8</td>
<td>0.5</td>
<td>Fasted</td>
</tr>
<tr>
<td>017</td>
<td>0.4</td>
<td>0.5</td>
<td>Fed</td>
<td>019</td>
<td>9.5</td>
<td>0.4</td>
<td>Fasted</td>
</tr>
<tr>
<td>020</td>
<td>4.5</td>
<td>1</td>
<td>Fasted</td>
<td>018</td>
<td>10</td>
<td>1</td>
<td>Fasted</td>
</tr>
</tbody>
</table>

Abbreviations: CH, carbohydrate; glucose-SIB test, glucose stable isotopes blood load test performed before breakfast (test 1), after lunch (test 2), or at a random time (test 3); GSDIa, glycogen storage disease type Ia; HV, healthy volunteer.

Extended metabolic assessment.
method in patients in whom glycogenolysis or gluconeogenesis is decreased (eg, ketogenic GSD subtypes, fructose-1,6-bisphosphatase deficiency) and as a confirmation tool to clarify the impact of genetic variants of unknown significance. Importantly, as this approach does not require intravenous infusion or collection of multiple venous blood samples it can be performed in outpatient settings. As such, this method overcomes organizational, financial, and psychological burden related to hospital admission, frequent venous sampling, and specific sample storing and processing.

Interestingly, in several of the 15 currently registered clinical trials for patients with GSDI, outcome parameters for efficacy have been selected that are counterintuitive or even dangerous, such as controlled fasting challenges. Novel

![Figure 3. Capillary and CGM glucose concentrations during glucose-SIB test 1 and glucose-SIB test 2. Capillary glucose concentrations (CBG) during glucose-SIB test 1 and 2 in patients with GSDIa (n = 100 [ie, 10 time points x 10 participants] per each glucose-SIB test) and HVs (HVs, n = 100 [ie, 10 time points x 10 participants] per each glucose-SIB test). Results are calculated compared with median baseline values calculated in each subgroup (ie, GSDIa and HVs, respectively) for each glucose-SIB test (100% = median of baseline values in each subgroup) and presented as median with range (grey circles show single participants’ values). 20% = 1 mmol/L glucose.

Table 3. Time-averaged glucose $R_a$ in patients with GSDIa and healthy volunteers

<table>
<thead>
<tr>
<th>Glucose $R_a$</th>
<th>Glucose-SIB test 1 (preprandial/fasted state)</th>
<th>Glucose-SIB test 2 (fed state)</th>
<th>Glucose-SIB test 3 (random fed state)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$mol/kg/minute</td>
<td>mg/kg/minute</td>
<td>mM/min</td>
</tr>
<tr>
<td>GSDIa attenuated</td>
<td>7.6-24.1</td>
<td>1.4-4.3</td>
<td>0.06-0.11</td>
</tr>
<tr>
<td>GSDIa severe</td>
<td>7.6-27.3</td>
<td>1.4-4.9</td>
<td>0.04-0.17</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>7.5-13.9</td>
<td>1.4-2.5</td>
<td>0.03-0.08</td>
</tr>
</tbody>
</table>

Abbreviations: GSDIa, glycogen storage disease type Ia; SIB, stable isotope blood.
For each glucose-SIB test the range of measured glucose $R_a$ is expressed in $\mu$mol/kg/minute, mg/kg/minute and mM/min, parameters that are commonly used in preclinical and clinical settings, respectively.
techniques are urgently needed to capture alterations in metabolic flux homeostasis that may better reflect therapeutic changes in pharmacodynamics and pharmacokinetics (41), such as CGM (7, 14), and stable isotope methods (9, 42). As glucose $R_a$ is directly linked to the underlying enzyme defect in GSDIa, it has the potential to develop into a primary disease activity biomarker as opposed to current biomarkers (43). The case-control application of the oral $\delta$-[6,6-2H$_2$]-glucose method may be develop into a tool to longitudinally monitor patients with GSDIa who discontinued nocturnal dietary

Figure 4. Glucose $R_a$ in the study participants. (A-C) Time-averaged glucose $R_a$ calculated with fixed $F$ and constrained $C$ and $K_a$. Mean and standard deviation are shown. (D) Glucose $R_a$ time course calculated with fixed $F$ and constrained $C$ and $K_a$. A line connecting the mean value calculated at each time point (thick line) and SD (shaded area) are shown (green, GSDIa attenuated; blue, GSDIa severe; red, HVs). * $P$ < .05.

Figure 5. (A) Correlation between the change in glucose $R_a$ and capillary blood glucose concentrations (CBG) during glucose-SIB test 1 in patients with GSDIa ($r = 0.79$, ** $P$ < .01). $\Delta$ was calculated as $|value\_end\_test - value\_start\_test| \times 100$. The baseline values were considered as the value start test; values at +120 (or +180) were considered as the value end test. (B-D) Relationship between calculated glucose $R_a$ and CBG during glucose-SIB test 1 in patients with GSDI with severe (B) and attenuated (C, D) phenotypes who developed hypoglycemia at the end of glucose-SIB test 1.
management after a so-called single shot innovative treatment (such as gene transfer or gene editing approach). In these individuals the contribution of the diet to the estimated glucose $R_d$ is expected to decrease because of less intense dietary management. Because current mRNA treatments require repeated in-hospital intravenous administrations, a modified approach by making use of short-term multitracer IV solution of [U-$^{13}$C]-glucose, [2-$^{13}$C]-glycerol, [1-$^2$H]-galactose, and acetaminophen may allow one to reliably estimate EGP and separately quantify contributions of gluconeogenesis and glycogenolysis (44, 45).

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Disclosures

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Data Availability

Data analyzed during the current study are available from the corresponding author on reasonable request.

Clinical Trial Information

NCT04311307 (registered March 17, 2020).

References