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REVISITING GLYCOGEN CONTENT IN THE HUMAN BRAIN

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Abstract

Glycogen provides an important glucose reservoir in the brain since the concentration of glucosyl units stored in glycogen is several fold higher than free glucose available in brain tissue. We have previously reported 3–4 μmol/g brain glycogen content using in vivo ^{13}C magnetic resonance spectroscopy (MRS) in conjunction with [1-^{13}C]glucose administration in healthy humans, while higher levels were reported in the rodent brain. Due to the slow turnover of bulk brain glycogen in humans, complete turnover of the glycogen pool, estimated to take 3–5 days, was not observed in these prior studies. In an attempt to reach complete turnover and thereby steady state ^{13}C labeling in glycogen, here we administered [1-^{13}C]glucose to healthy volunteers for 80 hours. To eliminate *any* net glycogen synthesis during this period and thereby achieve an accurate estimate of glycogen concentration, volunteers were maintained at euglycemic blood glucose levels during [1-^{13}C]glucose administration and ^{13}C-glycogen levels in the occipital lobe were measured by ^{13}C MRS approximately every 12 hours. Finally, we fitted the data with a biophysical model that was recently developed to take into account the tiered structure of the glycogen molecule and additionally incorporated blood glucose levels and isotopic enrichments as input function in the model. We obtained excellent fits of the model to the ^{13}C-glycogen data, and glycogen content in the healthy human brain tissue was found to be 7.8 ± 0.3 μmol/g, a value substantially higher than previous estimates of glycogen content in the human brain.

Keywords

^{13}C magnetic resonance spectroscopy; human brain; glycogen; mathematical modeling

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Introduction

Cerebral glycogen is found primarily in astrocytes in the adult brain [1] and has been implicated in supporting the function and survival of neurons during glucose deprivation [2,3], as well as during normal brain activity [4]. While it is generally accepted that the brain contains substantially lower levels of glucose stored in the form of glycogen than the muscle and liver [5], glycogen content of the mammalian brain has not been firmly established under normal physiology. Thus, most early rodent studies estimated glycogen content of the brain between 2 – 4 μmol/g using acid extraction methods [4,6–9] while substantially higher values of up to 11 – 12 μmol/g were reported more recently in carefully handled rats with minimal environmental stimuli and ethanol extraction [10]. The determination of brain glycogen content in invasive animal studies is challenging because tissue sampling and extraction procedures suffer from postmortem artifacts and incomplete enzyme inactivation [11]. On the other hand, high power (10 kW) focused microwave irradiation rapidly denatures enzymes and thereby revealed higher cerebral glycogen concentrations (6–8 μmol/g) in rats than most earlier extraction studies [12].

In vivo ^{13}C magnetic resonance spectroscopy (MRS) methodology was developed in late 1990s to assess cerebral glycogen metabolism noninvasively [13] and provided a glycogen content estimate of 3.3 μmol/g in the awake, normoglycemic rat following extended pre-labeling with ^{13}C glucose [14]. This method was further refined to eliminate label turnover as a variable in the determination of glycogen concentration and resulted in estimates of ~5–6 μmol/g glycogen content in the rat brain [15,16]. To date, ^{13}C MRS in conjunction with intravenous (IV) administration of [1-^{13}C]glucose to isotopically label glycogen remains the only method to detect glycogen and estimate its content and turnover rate in the living human brain [17,18]. Using this method, we have previously reported 3.5–4.3 μmol/g for glycogen content of the healthy human brain [19,20]. These prior studies also suggested that it would take 3–5 days to turn over the entire metabolically active glycogen pool, which was not achieved with the 6–50 hour infusion times utilized. Short infusion durations may affect fitting procedures of the experimental ^{13}C label incorporation into glycogen in a model-dependent manner, thereby biasing the estimated glycogen concentration (see below). Furthermore, slightly hyperglycemic blood glucose levels (110–130 mg/dl) were targeted in these prior studies during labeling of brain glycogen with [1-^{13}C]glucose to minimize endogenous hepatic glucose production and hence dilution of infused ^{13}C-labeled glucose and to maintain high isotopic enrichment (IE) levels in blood glucose [19]. Finally, we had used a metabolic model that assumed a homogeneous pool of glucosyl units in glycogen. This model entailed that glycogen labeling is described by a single exponential saturation function, where glycogen content is related (or equal, in case of 100% IE of glucose) to the asymptotic saturation point. However, the glycogen molecule is not homogeneous and its labeling could be better described by a sum of a certain (unknown) number of exponential saturation curves, each identifying a discrete component characterized by a specific glycogen fraction and its associated turnover. In particular, the glucosyl residues located in the core of the glycogen molecule have a slower turnover than those located on the molecular surface. Therefore, the former are invisible when experimental data are fitted against a single exponential saturation function. For this reason, glycogen content might be

underestimated using homogeneous modeling. This is especially relevant for short duration times, because the fit would capture only the components with fastest turnover.

The current study was designed to overcome these potential limitations of prior estimates of glycogen content in the human brain: First, we administered [1-^{13}C]glucose for 80 hours, the longest duration for metabolite labeling in humans to date, in an attempt to turn over the entire cerebral glycogen pool. Second, we targeted euglycemic blood glucose levels during the 80 hour infusion to avoid any net glycogen synthesis. Any fluctuations in plasma glucose concentration and glucose IE were taken into account in the modeling of the data. Finally, the data were fitted with a biophysical model that was recently developed to take into account the tiered structure of the glycogen molecule [21]. The analysis of label incorporation performed using this model allowed us to determine the time-dependent fraction of the glycogen molecule that is otherwise invisible. The overall goal was to provide an accurate in vivo estimate of glycogen content at normal physiology in the healthy human brain tissue.

Materials and Methods

Subjects

Five healthy male volunteers (age 41 ± 9 years, BMI 27 ± 2 kg/m^2) were studied after giving informed consent using procedures approved by the Institutional Review Board: Human Subjects Committee of the University of Minnesota.

Experimental Design

Volunteers presented to the Center for Magnetic Resonance Research for MR scanning. An IV catheter placed antegrade in a forearm was used for infusion and a catheter placed antegrade in the other arm was used for blood sampling. A bolus injection of 20 g of [1-^{13}C]glucose (Cambridge Isotope Laboratories, Andover, MA, USA, prepared as 20% weight/volume D-glucose in water with 50% IE) was administered. Twenty minutes later a continuous infusion of 2 mg kg^{-1} min^{-1} of [1-^{13}C]glucose (prepared as 20% weight/volume D-glucose in water with 25% IE) was started and then adjusted to maintain blood glucose at 90 mg/dl (5 mmol/l) based on samples of venous blood collected every 10–60 minutes and measured on an automatic glucose analyzer. Additional blood samples were collected for the later determination of serum insulin concentrations by a chemiluminescent assay (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA), and of the IE of the plasma glucose by gas-chromatography-mass spectrometry as described previously [22]. The infusion of ^{13}C-glucose continued for 80–81 hours, except in one subject in whom the infusion had to be stopped after 29 hours due to complications with the IV line. During the infusion subjects received breakfast, lunch and dinner with mid-morning, mid-afternoon and bedtime snacks if desired. Each meal and snack had no more than 30g of carbohydrates. Cerebral ^{13}C-glycogen levels were measured using MRS at ~8, 20, 32, 44, 56, 68 and 80 hours after the start of the infusion, except in one volunteer where the last ^{13}C-glycogen data point was obtained at 31 hours and the 44–80 hour measurements were missed due to loss of IV access.

MR Spectroscopy

^{13}C-glycogen levels in the brain were measured using methods described before [20]. Briefly, measurements were performed on a 4 T magnet (Oxford Magnet Technology Inc., Witney, UK) interfaced to an Agilent DirectDrive console (Agilent Technologies, Santa Clara, CA, USA) using a quadrature 14 cm ^{1}H surface coil combined with a 9 cm diameter linear ^{13}C coil [23]. The [1-^{13}C]glycogen NMR signal was localized in a 7 × 5 × 6 cm^3 voxel in the occipital lobe by 3-D outer volume suppression combined with 1-D image-selected in vivo spectroscopy [24]. Each data point presented was acquired over 30 minutes; the time points given for each data point are mid-way through the glycogen data acquisition. The amount of ^{13}C label in the C1 position of glycogen was quantified by the external reference method [25,24]. All ^{13}C-glycogen levels were corrected for the cerebrospinal fluid (CSF) content of the voxel as described before [26].

Modeling Glycogen Turnover

The biophysical model of the glycogen molecule used to fit experimental data takes into account the structural properties of cellular glycogen [21]. In particular, the accessibility of glycogen synthase and glycogen phosphorylase to glucosyl residues varies with the size of the glycogen molecule. The resulting turnover is highly heterogeneous, with external regions turning over more rapidly than internal ones. Thus, the probability of replacement of a glucosyl residue is not constant but depends upon its location. The main assumption of the model is that steric factors completely control enzyme activity, which is based on the notion that cessation of glycogen biosynthesis is brought about by the high density of glucosyl residues on the surface [27]. As far as the rate equations for glycogen synthase and glycogen phosphorylase are concerned, the approach is equivalent to first-order kinetics with non-linearity introduced by rate constants depending on glycogen size. The algorithm uses the rate equations to calculate enzyme activity at each time-step (here, 1 minute). Synthesis and degradation are then applied concurrently on the glycogen molecule through random selection of available reducing ends (synthase and phosphorylase cannot attack the same residue at the same time). According to the status of the chain being attacked, new branches are initiated by synthase or trimmed branches are transferred by phosphorylase, mimicking the action of glycogen branching and debranching enzyme, respectively. The concentrations of unlabeled and ^{13}C-labeled glucose, measured hourly (and more frequently in the first 4 hours) in plasma, were used as input function during the simulation, thus determining the relative probability of residue incorporation in the polysaccharide. Hence, ambient glucose level was time-dependent, which directly affects the rate of synthesis by glycogen synthase (glucose is assumed to be linearly related to the actual substrate of the enzyme UDP-glucose). In turn, any transient change in glycogen level brought about by altered synthase activity also enhances or suppresses the rate of degradation by glycogen phosphorylase (glycogen is the substrate of the enzyme). Changes in plasma glucose relative to euglycemia were taken into account by assuming a linear dependence of glycogen synthase on glucose concentration. This assumption is justified because in our experimental conditions the departures of plasma glucose from the euglycemic value (taken as 90 mg/dl = 5 mmol/l) were relatively small and the plasma glucose levels were within a euglycemic range (70–110 mg/dl = 3.9–6.1 mmol/l) for almost the entire duration of the infusion (Fig. 1a). Therefore, we can retain only the linear term of Taylor expansion in the relevant reaction rate equation

even though we do not know the exact profile of enzyme activity as a function of substrate. Notably, the model stores a complete structural representation of the entire glycogen molecule, so that the position of each glucosyl residue can be monitored. For consistency with previous experimental data, the model further assumes that glycogen synthase (i) preferentially works on inner chains and (ii) exhibits higher mobility than phosphorylase in translocating from an attacked chain to another [21]. The latter assumption is based on the constraint that phosphorylase attacks neighboring glucosyl chains with a probability of reaching internal residues that decreases exponentially with the distance from the surface of the molecule [21]. In other words, the model assumes distinct, stochastic activity patterns for glycogen synthase and glycogen phosphorylase, which depend on the location where enzymes operate in a given instant. The resulting label incorporation curves cannot be described by an analytical (exact) expression. It is noted that the structural information about the glycogen molecule enters into the model as a continuous variable, which is different from assuming the existence of discrete components, as illustrated above.

Total tissue glycogen content was determined by multiplying the total number of residues in the glycogen molecule (averaged over the 80 hours) by the coefficient obtained from least-square fitting with experimental data. Statistical errors on the glycogen estimate were calculated as standard deviation of the residuals. Goodness-of-fit was examined through reduced χ^2 (i.e. squared sum of residuals divided by degrees of freedom).

All simulations were carried out using SWI Prolog (University of Amsterdam, The Netherlands; http://www.swi-prolog.org/). Statistical analysis of simulated versus experimental data was performed using Matlab (The Mathworks, Inc., Natick, MA, USA; http://www.mathworks.com/).

Results

To monitor glycogen metabolism and estimate the concentration of the metabolically active glycogen pool in the human brain, we infused [1-^{13}C]glucose intravenously to 5 healthy volunteers. The IV lines remained patent in 4 volunteers for over 80 hours, while the infusion had to be stopped after 29 hours in one volunteer. In all volunteers, we were able to maintain normoglycemia throughout the infusion, with a between-subject mean plasma glucose concentration of 99 ± 6 (SD) mg/dl = 5.5 ± 0.3 mmol/l (Fig. 1a). The insulin levels were also in the normal physiologic range (between-subject mean 29 ± 9 mU/l), with postprandial increases and lowest levels overnight (Fig. 1b). As expected, we observed higher fluctuations in plasma glucose IE (Fig. 1c, between-subject mean 21 ± 2%) than our prior studies [19,20,28], but we were able to incorporate these fluctuations in our modeling (see below).

^{13}C-glycogen levels increased until the end of the infusion, with some indication of leveling off (Fig. 2a). Note that these ^{13}C-glycogen levels were corrected for the CSF contribution to the voxel (between-subject mean 12 ± 5%) and therefore represent concentrations in the brain tissue. To roughly estimate what the highest ^{13}C-glycogen levels we detected at 80 hours corresponded to in terms of labeled + unlabeled glycogen, we calculated newly synthesized glycogen levels, i.e. glycogen turned over during the infusion. These are

obtained by dividing ^{13}C-glycogen concentrations by each subject's mean glucose IE, hence take into account unlabeled glucosyl units that are incorporated into glycogen in parallel to ^{13}C-glucose, and do not refer to net synthesis. Based on this calculation, all 4 subjects who received [1-^{13}C]glucose for 80 hours had at least 6 μmol/g cerebral glycogen (Fig. 2b).

Next, we fitted the data to the biophysical model of the tiered glycogen molecule, using only the data from the 4 volunteers in whom we achieved 80 hour infusions. Since glycogen turnover (expressed in units μmol/(g·h)) and total glycogen content (expressed in units μmol/g) are interdependent variables, we first tested how well different basal (i.e. at euglycemia, or 90 mg/dl) rates of molecular turnover ranging from 10 to 80 glucosyl residues per minute fit the data. We found good correspondence between experimental and simulated time courses for label incorporation into glycogen at single subject-level, especially for molecular turnover rates higher than 20 residues per minute (Fig. 3a–d). The improved performance achieved with higher simulated molecular turnover rates in following experimental data reflects the better adaption to changes in ambient glucose levels and IEs.

To quantitatively determine the most appropriate molecular turnover rate, we calculated the reduced χ^2 of residuals from each least-square fitting as a measure of goodness-of-fit (Fig. 3e). Simulated data supported a basal molecular turnover rate of 30–50 residues per minute, which is consistent with our previous estimate of 40 residues per minute [21,19]. Single-subject determination of total glycogen content based on simulated data showed decreasing values at increasing rates of molecular turnover (Fig. 3f–i). For a molecular turnover rate of 40 residues per minute, we obtained a final estimate for brain glycogen content of 7.8 ± 0.3 μmol/g wet weight. These values translate to a rate of turnover for brain glycogen of 0.35 ± 0.01 μmol/(g·h). Note that the 0.35 μmol/(g·h) value reflects the molecular turnover (e.g. 40 residues/min), which is independent of the tiered molecular structure.

We also found that infusion duration affects the estimate of glycogen content (Fig. 3j). Specifically, a fast turnover rate results in underestimation of glycogen, and vice versa, with short infusions. However, in the case of a turnover rate of 40 residues per minute the effect of infusion duration was not significant.

To examine the effect of the inclusion of physiological parameters (plasma glucose concentration and IE) on model outcomes, we performed further simulations without these additional inputs. Fig. 4 shows the results of this analysis for the subject with the largest fluctuations in glucose IE (subject #2), chosen to clearly demonstrate these effects. Large fluctuations of simulated ^{13}C-glycogen concentration (Fig. 4b) resulted from the incorporation of glycemic level (Fig. 4c) and IE (Fig. 4d). As expected, the incorporation of plasma glucose and IE into the model substantially improved the fit, as evidenced by the lower discrepancy between simulated and experimental data (Fig. 4e). Cross-correlation between data simulated in the presence and absence of physiological parameters (Fig. 4f) showed delayed positive correlations between the ^{13}C-glycogen fluctuations and IE, whereas there was no significant correlation with plasma glucose concentration. At group-level, the cross-correlation coefficient for IE was 0.53 ± 0.13 and the associated delay for IE was 4.2 ± 1.2 h (mean ± SD).

Finally, in order to determine the amount of glycogen that turned over in 80 hours, we performed additional simulations by assuming 100% glucose IE. Based on these simulations, with a turnover rate of 40 residues per minute, we found that 68% of the glucosyl units in the glycogen molecule turn over in 80 hours. Thus, approximately one third of the glucosyl residues in the glycogen molecule are not reached by glycogen metabolizing enzymes during this time frame. Note however, that it is not possible to determine which tiers are not reached based on the 68% turnover value, i.e. what the inner core corresponds to in terms of tier number, because the process of turnover is stochastic. In particular, the probability for the inner core to turn over is negligible but not zero (see Materials and Methods). If 21% of the ambient glucose molecules carry the ^{13}C label on average during the glycogen labeling period (21% corresponds to the average glucose IE measured in the present set of experiments) and if 68% of glycogen is turned over during the same period, this would result in ^{13}C labeling of 13% of the glucosyl residues in glycogen (approximately 68% × 21%) (Fig. 5).

Discussion

Here we investigated cerebral glycogen content in the healthy human brain under normal physiology by administering ^{13}C-glucose intravenously over 3+ days and monitoring its incorporation into cerebral glycogen with ^{13}C MRS. Using an approach that overcame potential confounds of prior studies, we found substantially higher cerebral glycogen levels (6–8 μmol/g) than we previously reported in the human brain (3–4 μmol/g) [19,20]. In addition, using a biophysical model of glycogen structure, we estimated that the entire glycogen pool was still not turned over after 80 hours due to the inaccessibility of the innermost tiers to glycogen metabolizing enzymes.

In order to obtain an accurate estimate of cerebral glycogen content, we implemented several differences in the experimental design of the current study vs. prior studies: First, we labeled glycogen for over 3 days and at euglycemia to avoid the possibility of net synthesis (above baseline levels). With these extended infusions, we were able to conclude that the healthy brain contains at least 6 μmol/g glycogen (Fig. 2b), even before mathematical modeling of the data. Shorter infusion periods in prior work may have resulted in underestimation of total glycogen (Fig. 3j), although this effect was minimal for a molecular turnover rate of 40 residues per minute. Second, the ^{13}C-glycogen levels in the prior studies were not corrected for CSF contribution to the MRS voxel [19,20]. Assuming similar CSF content in the voxel in those studies (12%) would increase the glycogen content estimate from 3.5–4.3 to 4–4.9 μmol/g, therefore this correction only partially explains the difference in the cerebral glycogen content estimated in the current study vs. prior reports. Finally, we utilized a model of tiered glycogen structure rather than the prior simple kinetic model that assumed homogeneous turnover behavior for all glucosyl units in glycogen [19,20]. We further noted that the newly synthesized glycogen levels we observed in the current study were somewhat higher than those at the same time points in prior work, e.g. compare Fig. 2b to Fig. 6 in [20] (after euglycemia series). Among the prior data sets, the data obtained under the supercompensation study in [20] was most similar to the current data in terms of physiology (blood glucose and insulin levels) and subject characteristics (mean age and BMI). To overcome the limited sample size in the current study and investigate the effect of

the lower newly synthesized glycogen levels obtained previously, we fitted the data from the supercompensation study in [20] (with estimated CSF correction) with the tiered glycogen model. This analysis resulted in 5–6 μmol/g cerebral glycogen concentration, still ~40% higher than previously estimated with the homogeneous glucosyl pool model. Therefore we can conclude that the main contributor to the higher glycogen content observed in the current study is the improved model that takes into account the tiered glycogen structure. Hence, the present approach takes into account the fact that the inner tiers of the glycogen molecule may never become enriched. Between the results obtained here and the fit of the model to the data from [20], it is clear that the human brain contains ~6–7 μmol/g glucosyl units stored in glycogen. This higher value for human brain glycogen content is consistent with recent reports in rodents [12,15,16], and even with the report of 11–12 μmol/g [10], considering blood glucose levels ranged from 9.2–9.3 mmol/l in that study (vs. ~5.5 mmol/l here) and that hyperglycemia is known to increase cerebral glycogen content.

We also obtained a higher glycogen turnover rate in this study compared with our previous estimates based on homogeneous modeling [19,20] although the turnover time constant of ~22 h (7.8 μmol/g / 0.35 μmol/(g·h)) obtained in the current study was identical to our prior observations [19,20], confirming the 3–5 day requirement for glycogen turnover. The turnover rate we detected in the human brain is lower than the range (0.5–0.9 μmol/(g·h)) that has been reported in rodent studies [9,13,16], which is fully expected since metabolic rates are substantially higher in rodents than humans. In addition, the current turnover rate is still ~1.5% of the cerebral metabolic rate of glucose (CMR_{Glc}) in the human brain (24 μmol/(g·h)) [22]. Note however that this rate refers to basal turnover averaged in a relatively large, resting brain area. Glycogen mobilization is a rapid process, which might generate substantial effects within seconds in localized and possibly very small regions. Thus, rapid, transient and localized increases in glycogenolysis may contribute little to overall glycogen turnover in the entire tissue (partial volume effect). Importantly, the relevance of brain glycogen in brain function, such as in memory formation [29,30] is likely region-dependent. Similarly, a low rate of glycogen turnover relative to glucose utilization during resting condition does not imply non-essential function, as glycogen is implicated in specific processes (for example K^+ uptake [31]) that cannot be rescued by glucose.

We further showed that IE is a major determinant for the kinetics of label incorporation into glycogen, and that nearly 4 hours are necessary for changes in IE to manifest as alterations in ^{13}C-glycogen level (Fig. 4). On the other hand, blood glucose levels did not correlate with fluctuations in glycogen labeling, which is expected since they covered a narrow range around euglycemia.

The primary limitation of our study is the small sample size dictated by the substantial expense associated with these studies and the several-day long experimental design [17,18]. Similarly, we studied an all-male cohort due to challenges with recruitment for these long experiments; however we have not detected any appreciable differences between males and females in our prior studies investigating glycogen metabolism [19,20,28]; therefore the conclusions obtained in this cohort should be generalizable to both genders. While the biophysical model we utilized was more realistic for investigating the turnover of the tiered glycogen molecule than our prior kinetic model [19], it also had the limitation that it is

designed to simulate an individual glycogen molecule. Therefore, the estimate of total glycogen content at the tissue level (i.e. containing a large number of molecules) is based on the assumption that the glycogen pool is homogeneous, which entails that all molecules are kinetically equivalent *on average*. However, glycogen granules can be polydisperse [32] and possibly compartmentalized [33] in vivo. Polydispersity and compartmentalization might invalidate the assumption of homogeneity, as distinct glycogen pools may be in different states of synthesis and degradation. However, the agreement between experimental and simulated data indicates that the homogeneous pool assumption is justified by steady-state conditions and long experimental durations, as discussed previously [21].

In conclusion, the healthy human brain tissue contains ~6–7 μmol/g glycogen, which is substantially higher than previously reported, but consistent with recent studies in rodents. Considering glycogen is only localized in glial cells, this implies high cellular levels of glycogen, further underlining its functional importance for cerebral metabolism.

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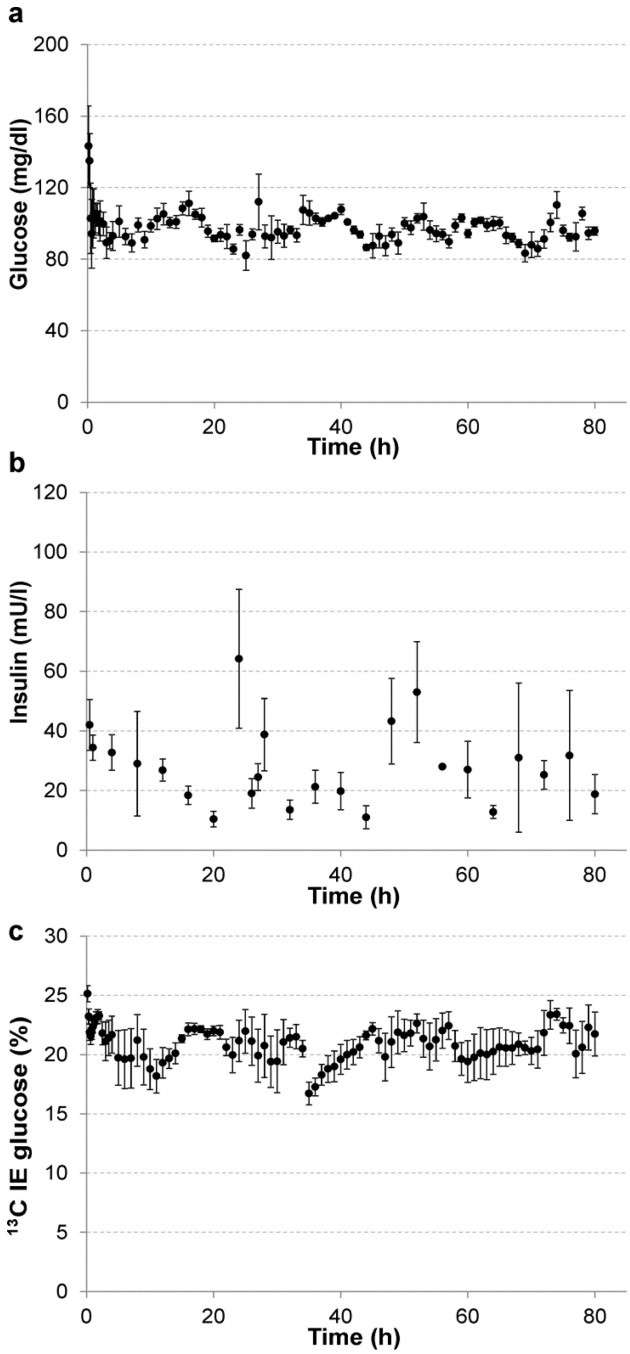

Figure 1.

Time courses of average (± SEM) plasma glucose (**a**), serum insulin (**b**) and plasma ^{13}C isotopic enrichment (IE) (c) levels during IV infusions of [1-^{13}C]glucose in 5 healthy volunteers (4 subjects received infusion for ~80 hours and the fifth subject received infusion for 29 hours). For glucose concentrations, 80 mg/dl = 4.4 mmol/l, 100 mg/dl = 5.6 mmol/l, 120 mg/dl = 6.7 mmol/l.

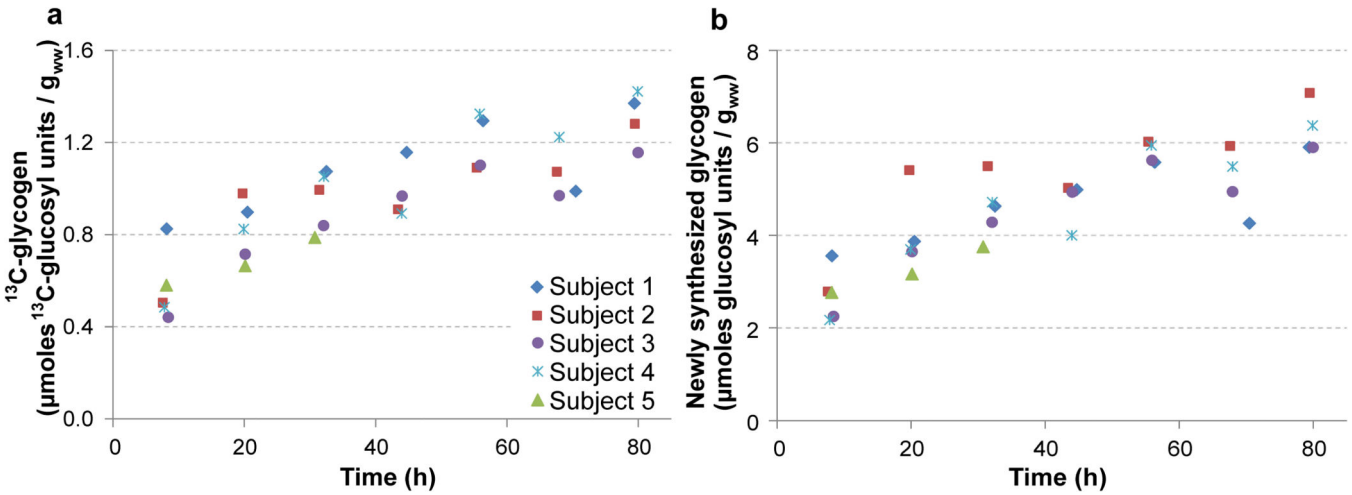

Figure 2.

^{13}C-labeled glycogen (**a**) and newly synthesized glycogen (**b**) concentrations in all volunteers. Newly synthesized glycogen levels are obtained by dividing the [1-^{13}C]glycogen concentrations by the mean plasma glucose IE of each volunteer. Note that because of the slow turnover of core glycogen the total glycogen content is higher than newly synthesized glycogen.

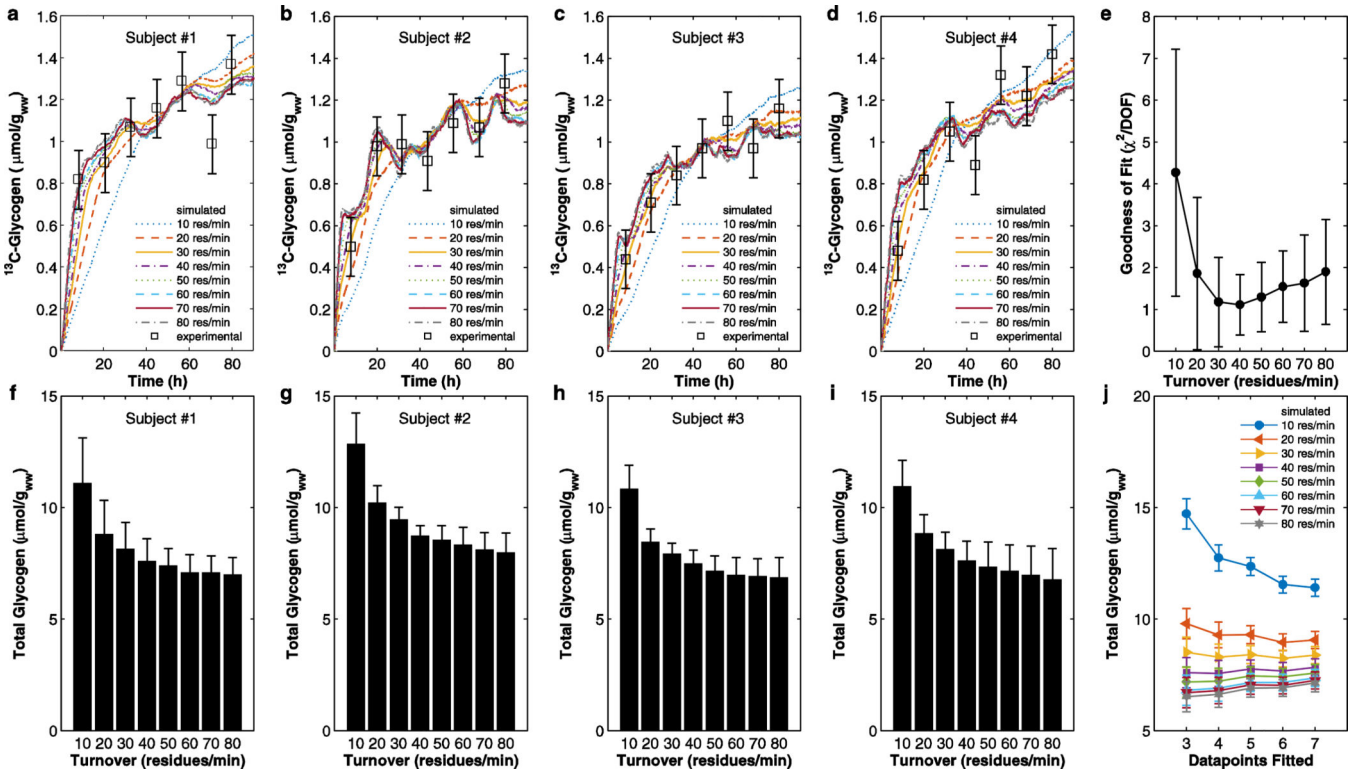

Figure 3.

Quantification of total glycogen content in the human brain from experimental measurements and simulated time-courses of ^{13}C-glycogen concentration during 80 hour long infusions in 4 healthy subjects. (**a–d**) Least-square fitting between experimental data and molecular-level simulations at different turnover rates. The error bars on experimental data have been estimated at 0.15 µmol/g. Molecular turnover rates of 10–20 residues per minute are found to be too slow for ^{13}C-glycogen to follow changes in plasma glucose and IE, whereas higher turnover rates result in larger fluctuations of simulated data. (**e**) Statistical χ^2 goodness-of-fit test. Across-subjects averages (mean ± SD) of reduced χ^2 as a function of molecular turnover. The best outcome is obtained for a turnover of 40 residues per minute, as evidenced by the value around unity of the reduced χ^2 (i.e. taking into account the degrees of freedom, DOF) and associated error. (**f–i**) Estimates of total brain glycogen as a function of molecular turnover, obtained by rescaling the fraction (i.e. labeled/total) of molecular-level label incorporation to the ^{13}C-glycogen concentration after the least-square fitting. Note that such scaling is possible because the structural model accounts for the portion of the molecule that does not undergo turnover. The estimate decreases at increasing molecular turnover rates and converges at a plateau for turnover greater than 50–60 residues per minute. The error in the estimate was calculated as the SD of the residuals (differences between experimental and simulated values). Note the smallest error in the estimate is obtained with a molecular turnover of 40 residues per minute. (**j**) Dependence of the estimate of total glycogen content upon the length of the experiment, i.e. the number of experimental data points used for modeling. The effect of experimental duration is substantial for molecular turnover rates of 10–20 residues per minute and less so for the highest simulated values of molecular turnover (60–80 residues per minute). For

intermediate values of molecular turnover (30–50 residues per minute) the effect of data points fitted on the estimate of total glycogen is negligible.

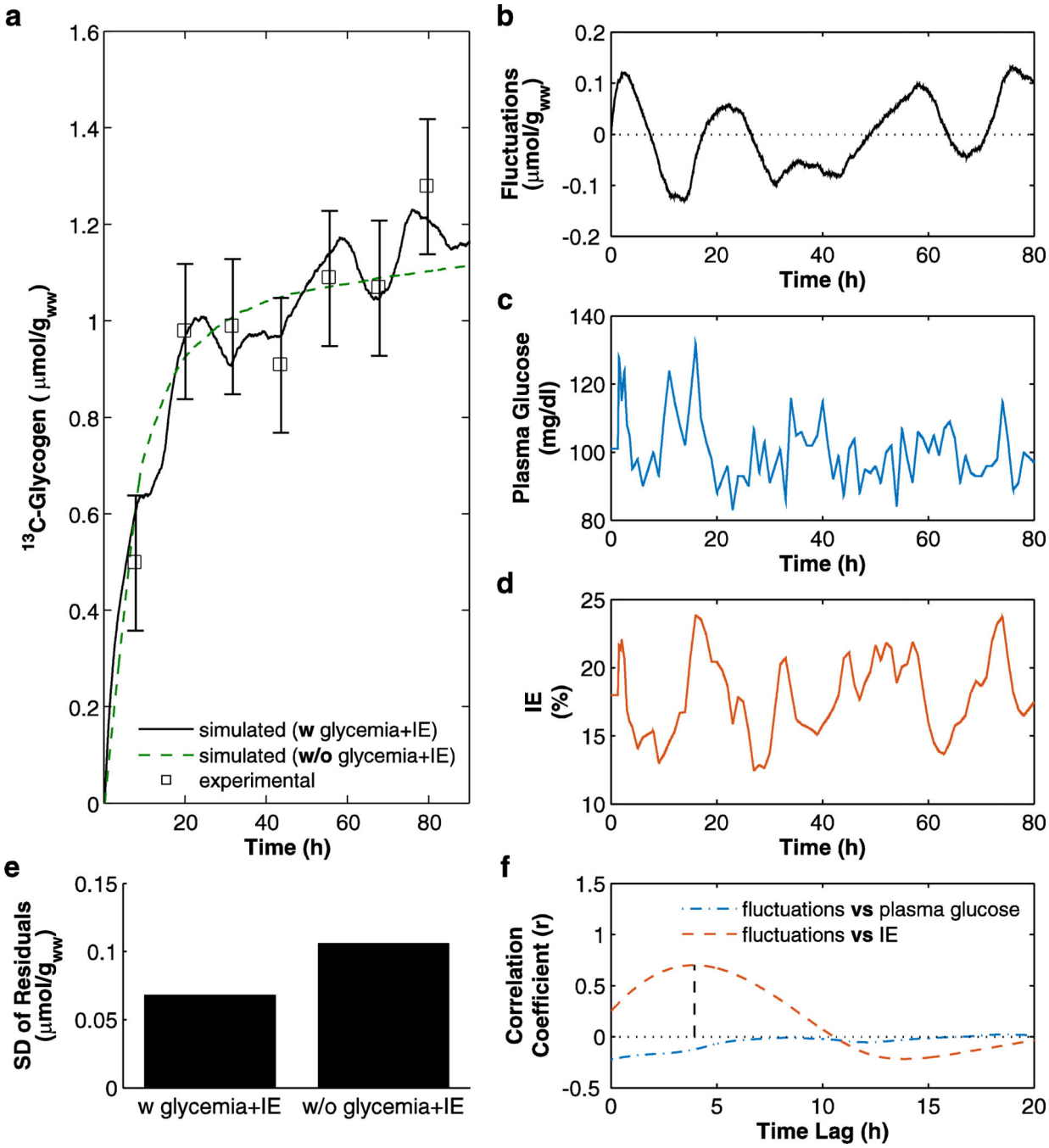

Figure 4.

Final simulation in one volunteer (subject #2) with molecular turnover rate of 40 residues per minute to demonstrate how the simulation is able to track the ^{13}C-glycogen levels. (**a**) Least square fitting between experimental measurements and data simulated either with or without the incorporation of plasma glucose (glycemia) and IE as additional inputs to the model. (**b**) Fluctuations of ^{13}C-glycogen obtained by subtracting the two time courses (shown in a) simulated with time-dependent or constant glycemia and IE. (**c–d**) Experimentally measured physiological parameters used as input functions to the model. (**e**)

Incorporation of physiological parameters results in better fit, as evidenced by the smaller standard deviation of residuals (difference between experimental and simulated data). (**f**) Cross-correlation between ^{13}C-glycogen fluctuations and physiological parameters. IE shows a delayed, positive correlation with the simulated ^{13}C-glycogen time-course, while changes in plasma glucose in the range utilized here do not affect ^{13}C-glycogen levels.

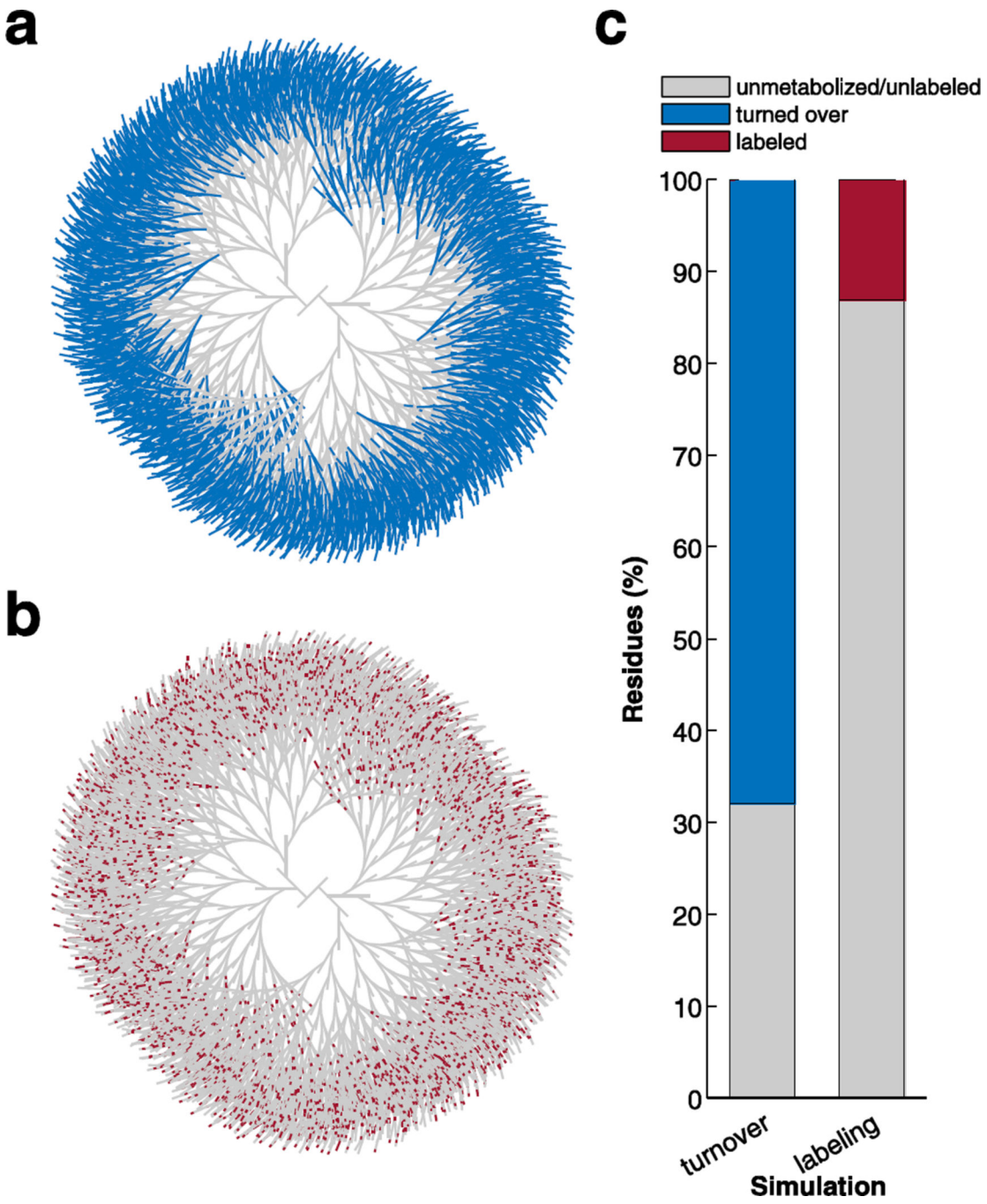

Figure 5.

Simulated data for turned over and ^{13}C-labeled glycogen at the end of an 80 hour ^{13}C-glucose administration. (**a**, **b**) Pictorial representations of mature glycogen molecules with highlighted turned over glucosyl residues (shown in blue in **a**) and ^{13}C-labeled residues (shown in maroon in **b**), and corresponding quantification (**c**). Simulations were obtained using a molecular turnover rate of 40 residues per minute. IE was set to 21% in the labeling simulation. During the 80 hour infusion period, nearly 68% of glycogen has been turned

over, while label retention was 13%. Note that these simulations were performed by assuming constant plasma glucose and IE.