Measuring pharmacodynamics in early clinical drug studies in multiple sclerosis

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KINETICS OF MYELIN BREAKDOWN PRODUCTS: A LABELING STUDY IN PATIENTS WITH PROGRESSIVE MULTIPLE SCLEROSIS

Submitted to: MS and demyelinating disorders

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ABSTRACT

BACKGROUND The majority of the available disease modifying therapies for Multiple Sclerosis (MS) reduce inflammation. These therapies do not yet target remyelination and neuronal degeneration, which is a major contributor to progressive disability in MS. The development of remyelinating therapies will benefit from a method to quantify myelin kinetics in patients with MS. In this study we labeled myelin in vivo with deuterium, which allowed us to model the kinetics of the myelin breakdown products β-galactosylceramide (β-GALC) and N-Octadecanoyl-sulfatide (NO-Sulf).

METHODS Five patients with MS, and six healthy subjects received 120 mL 70% D_2O daily for 70 days to label β-GALC and NO-Sulf. Mass spectrometry and compartmental modeling were used to quantify the turnover rate of β-GALC and NO-Sulf in cerebrospinal fluid. The turnover rate of patients was compared to changes in MRI Magnetization Transfer-ratio of both normal appearing white matter and lesions.

RESULTS The turnover rate constants of the fractions of β-GALC and NO-Sulf with non-negligible turnover were 0.00186 and 0.00714 in MS patients, which corresponds to a turnover half-life of 373 days and 96.5 days, respectively. The effect of MS on the NO-Sulf (49.4% lower fraction with non-negligible turnover) was more pronounced compared to the effect on β-GALC turnover (18.3% lower fraction with non-negligible turnover).

CONCLUSIONS The kinetics of myelin breakdown products in the CSF are different in patients with MS compared with healthy subjects. This can be caused by slower myelin production in these patients, by a higher level of degradation of a more stable component of myelin, or, most likely, by a combination of these two processes.

Deuterium labelling in combination with lumbar punctures is a useful method for quantification of metabolic processes of the central nervous system. This method can be used to quantify myelin turnover in patients with progressive MS and can therefore be used in proof of concept studies with remyelination therapies.

BACKGROUND

Multiple sclerosis (MS) affects millions of individuals worldwide and is the most common autoimmune disorder of the central nervous system (with estimated 2 million patients)[1]. Despite the fact that the disease has been known for more than 180 years, the exact pathophysiology of the disease is not clear[2,3]. MS is primarily an inflammatory disorder of the brain and spinal cord in which focal lymphocytic infiltration leads to damage of myelin (demyelination) and axons[4]. The majority of the available disease modifying therapies for MS reduce inflammation. However, these therapies do not enhance remyelination or inhibit neuronal degeneration, which is a major contributor to progressive disability in MS[5].

Development of compounds that stimulate remyelination is seen as an important target for drug development. Examples of such compounds are anti-LINGO antibodies[6], RHIGM22 antibodies[7], Olesoxime[8], and Quetiapine fumarate[9]. The most direct way to demonstrate pharmacological effects of these compounds in future proof-of-concept studies is the quantification of myelin formation or remyelination.

Currently, there are several methods to quantify remyelination in humans such as MRI magnetization transfer imaging (MTI) and diffusion-weighted imaging[10]. Also, non-MRI methods like Positron Emission Tomography using [^{11}C]PIB[11], or Visual Evoked Potential[6] can be used. Although these techniques are improving in accuracy, there is still a need to develop more sensitive and specific methods[12], especially since the development of remyelinating and neuroprotective therapies is increasing[13].

We recently demonstrated that myelin components could be labeled in healthy subjects using deuterium, which allowed us to model the kinetics of the myelin breakdown product β-galactosylceramide (β-GALC). This approach offered a method to quantify possible changes in myelin kinetics after treatment with a remyelinating compound[14].In addition to β-GALC, N-Octadecanoyl-sulfatide (NO-Sulf) was also measured. Both lipids are present abundantly in the brain of vertebrates, comprising almost one-third of the lipid mass of myelin and can be regarded, when present in the CSF, as being specific for CNS myelin[15]. MRI scans were performed to compare our findings with imaging correlates of demyelination[16].

In the current study we used the same method as in our previous study to measure and model kinetics of myelin breakdown products in patients with progressive multiple sclerosis. The kinetics of myelin formation and breakdown have so far never been measured in vivo in patients with MS.

METHODS

Subjects

Patients included had a diagnosis of primary progressive or secondary progressive MS according to the revised McDonald criteria[17] for at least 1 year. Patients had to be between 18 and 70 years old and have a body mass index of 18 to 30 kg/m^2 and had all given written informed consent. All subjects underwent a full medical screening, including medical

history, a physical examination, blood chemistry, hematology and virology (hepatitis B, C and HIV) urinalysis and electrocardiogram (ECG) to assess eligibility. Key exclusion criteria were: contraindications for lumbar puncture, contraindication of an MRI, clinically significant abnormalities during screening and a relapse in one month before start of the study.

Study design

During the study, six patients with progressive multiple sclerosis were instructed to consume 120 mL of 70% D_2O daily for 70 days. Weekly urine samples were collected and used to measure the percentage of D_2O in the subject's total body water. All subjects underwent five lumbar punctures (LPS) for CSF collection on days 35, 70, 94, and 167, and a final LP 1-1.5 years after the start of the study. Blood was collected for safety measurements (routine hematology and chemistry). The patients had two MRIS: one between day 0 and 35 (before the first LP) and one on day 167 (fourth LP), to compare changes in imaging biomarkers related to myelination tothe deuterium labeling results.

Published and unpublished data from our previous study in healthy subjects[14] were used not only to quantify the kinetics of myelin breakdown in patients, but also to compare them to healthy subjects through simultaneous modelling.

Study approval

The study was approved by the Medical Ethics Committee of the BEBO Foundation (Assen, The Netherlands). The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki.

Measurement of deuterium enrichment in the total body water

This method has been described previously[14,18,19]. D_2O content in total body water was measured using a method which relies on the base-catalyzed exchange of hydrogen (deuterium) between water and acetone. ^2H-labeling of $^{13}C_3$ acetone is then determined using gas chromatography/mass spectroscopy (GC-MS) analysis. The deuterium fraction in the urine sample was calculated using the ratio of singly deuterated to non-deuterated forms. Measuring D_2O in urine has been established as reliable marker of body water D_2O, and has been used in other studies[20-22]. Urine was used because its collection is least invasive for subjects participating.

Measurement of deuterium enrichment of lipids in CSF

Isotopomer data of β-GALC and NO-Sulf were obtained using a tandem mass spectrometry method described previously[14]. The isotopomer data was used to calculate the change in average number of additional neutrons (the replacement of a hydrogen atom by a deuterium atom adds one neutron to the molecule). This was then divided by the total number of measurable hydrogen atoms, which is 78 for β-GALC and 68 for the NO-Sulf. This yields the change from baseline of the average deuterium fraction of the hydrogen atoms of the lipids. N-Octadecanoyl-sulfatide results from the healthy subject cohort were not reported in the previous study but are reported in the current report.

Compartmental model

A compartmental model that describes the turnover of body water (precursor) and the myelin lipid (product) was fitted to the enrichment data of β-GALC and NO-Sulf using the non-linear mixed effects modeling program NONMEM, version 7.3.0[23]. The first-order conditional estimation with interaction method was used to estimate typical parameter values and their inter-individual variability.

The body water turnover was characterized with two structural parameters: the daily water input/output, and the size of the total body water pool. A theoretical estimate of subjects' total body water—based on their sex, height, weight and age—was used as a covariate for daily water input/output[24]. This model described the deuterium enrichment of body water over time.

A schematic overview of the model is shown in figure 1. The production of the myelin lipids β-GALC and NO-Sulf from the (indirect) precursor body water was characterized with two structural parameters: fraction of the myelin lipid with a fast turnover, and the turnover rate of that fraction. The turnover rate of the remaining fraction of lipid (with a stable turnover) was assumed to have a negligible impact on the deuterium enrichment of the lipid in this study, and was therefore not estimated (i.e., assumed to be zero). The model parameters for β-GALC and NO-Sulf were estimated independent from each other.

With these models, data from both healthy subjects and patients with MS were analyzed in a pooled dataset. A potential effect of MS on the parameters of the lipid turnover model was explored in the model. An estimated effect of MS was included in the final model if it significantly ($p<0.05$) improved the model fit compared to a model in which the same parameter value was used for healthy subjects and patients. Minus two times the log likelihood was used to assess the model fit.

MRI

To study changes in tissue integrity in the normal appearing white matter, high resolution structural MRI (3D-T1 and FLAIR) and magnetization transfer imaging (MTI) scans were performed. 3D-T1-w scans were made to segment white matter from CSF and from grey matter. FLAIR images were used to segment white matter lesions from the white matter. The MTI-ratio (MTR) was calculated in the remaining normal appearing white matter and lesions separately[25]. MRI was performed before the first LP, and a second one 167 days after start of the study. All imaging was performed on a whole-body MR system operating at 3 Tesla field strength (Philips Medical Systems, Best, The Netherlands). The following parameters were used: 3DT1-weighted images: TR = 9.7 ms, TE = 4.6 ms, FA = 8°, FOV = 224 × 177 × 168 mm, resulting in a nominal voxel size of 1.17 × 1.17 × 1.4 mm, covering the entire

brain with no gap between slices, acquisition time was approximately 5 minutes. FLAIR: TR = 11000 ms, TE = 125 ms, FA = 90°, FOV = 220 × 176 × 137 mm, matrix size 320 × 240, 25 transverse slices with a slice thickness of 5 mm with no gap between slices. MTI imaging: TR = 100 ms, TE = 11 ms, FA = 9°, FOV = 224 × 180 × 144 mm, matrix size 224 × 169.

RESULTS

Clinical study in patients

Six patients with progressive MS were enrolled in the study, four of them completed the study. Both drop-outs were replaced, but only one of the replaced patients completed the study. Only data from patients who completed the study were analyzed. Demographics are summarized in table 1. Missing data in the analysis are the LP 4 for subject 7 (LP failed) and LP 5 for subject 8 (subject did not participate). Most of the reported adverse events were mild and transient. One Serious Adverse Event was reported: subject 8 was admitted to the hospital 4 months after start of the study, with a diagnosis of urinary tract infection that required intravenous antibiotic treatment. This was assessed as unlikely related to the administration of D_2O. Most frequent reported adverse events were fatigue (60%) and dizziness (60%). Post-dural puncture headache was not reported.

Body water enrichment

Both patients and the healthy subjects described in the previously published study[14] reached steady state deuterium enrichment in body water during the 70-day labeling period (see table 2 and figure 2). There was considerable inter-individual variability in the extent of body water enrichment, with maximum values ranging from 0.017 to 0.037. Body water enrichment decreased rapidly after the last D_2O dose, as expected.

β-GALC enrichment

The maximum deuterium enrichment of β-GALC in patients was about 27-fold lower than that of body water and ranged from 0.00047 to 0.0015. Comparable to what was observed in the healthy subject cohort, the enrichment of β-GALC decreased slowly in the patients with MS: at day 167, the β-GALC enrichment was still high (between 71% and 100% of maximum observed enrichment) and 1.5–2.0 years after the start of the study, a measurable β-GALC enrichment above baseline was still present in all subjects: between 24% and 60% of maximum observed enrichment (figure 3).

NO-Sulf enrichment

The maximum enrichment of NO-Sulf was about 2-3-fold higher than β-GALC, and 10-fold lower than that of body water. Maximum enrichment ranged from 0.0010 to 0.0018 in healthy subjects, and from 0.0026 to 0.0041 in MS patients (see table 2 and figure 4).

Compartmental model output

Inter-individual variability in the precursor model (body water) was only estimated for the parameter that represents daily water input/output; for the other structural parameters it did not result in a significantly improved model fit. The kinetics of the deuterium enrichment were then used as input for the models describing the turnover of β-GALC and the NO-Sulf. The parameter estimates of the model are given in table 2. The final model resulted in an adequate characterization of the observed deuterium enrichment data in body water β-GALC and the NO-Sulf (figure 2, figure 3 and figure 4).

The typical values for the daily water input/output (3.39 L) and the size of the total body water pool (38.9 L) correspond to a turnover rate constant of 0.087 or a body water half-life of 8.0 days. The turnover rate constants of the fractions of β-GALC and NO-Sulf with non-negligible turnover were much lower: 0.00186 and 0.00714, which corresponds to a turnover half-life of 373 days and 96.5 days, respectively. The fraction of β-GALC that originates from the fast or non-negligible turnover rate, was estimated at 34.4%. Limited variability between individuals was observed in the value of this parameter, for which a coefficient of variation of 7.4% was estimated with poor precision (relative standard error of estimate of 93%). The fraction of the NO-Sulf with a non-negligible or fast turnover was estimated to a similar value of 36.6%, although a higher degree of unexplained variability between individuals was observed there (CV=24.3%).

We observed a significant difference in the turnover of both myelin lipids between the healthy subjects and the MS patients: for β-GALC 0.817 (95% confidence interval of 0.71 to 0.93, p-value: <0.01) and for NO-Sulf 0.506 (95% confidence interval of 0.35 to 0.66, p-value: <0.005). The effect of MS on the myelin lipid turnover was included in the model as an effect on the parameter that represents the estimated fraction with a non-negligible turnover. This resulted in a slightly better statistical fit of the data, when compared to model where the effect was included on the turnover rate of the fast fraction. The effect of MS on the NO-Sulf (49.4% lower fraction with non-negligible turnover) was more pronounced compared to the effect on β-GALC turnover (18.3% lower fraction with non-negligible turnover). Figure 5 shows model predictions for a typical healthy subject and a typical MS patient for β-GALC and NO-sulf, if they would participate in a study with the same design: 120 mL heavy water (70% D_2O) daily, for 70 consecutive days. Without the administration of water, no deuterium would be incorporated, and the deuterium fraction would stay zero (same value as baseline).

MRI

The normalized peak height (norPH) of the normal appearing white matter and of the white matter hyperintensity lesions was calculated for all patients at both time points. The MTI norPH in the normal appearing white matter decreased in 4 patients (with 16.6, 8.3, 9.1 and 10.8%) and increased in one patient (5.5%). For three subjects, the norPH of identified lesions increased in the period between the two scans (+8.7, +16 and +21%), the other two subjects had an decrease in norPH of the lesions (-12 and -14%) (table 3).

DISCUSSION

This is the first study to measure the kinetics of deuterium-labelled myelin breakdown products beta-galactosylceramide and N-octadecanoyl-sulfatide in the CSF of patients with progressive MS. In a pooled compartmental analysis, we analysed data from these patients together with healthy subject data from a previous study[14]. This analysis showed that the level of deuterium incorporation in myelin breakdown products was lower in MS patients than in healthy subjects, which indicates that myelin formation was slower, or breakdown of a more stable myelin component was higher.

The lower levels of deuterium incorporation that we observed in patients with MS, were then described in a model, which estimated a lower fraction with non-negligible turnover in the MS patients compared to the healthy subjects: 49.9% lower for NO-sulf, and 18.3% lower for β-GALC. There are several possible physiological explanations for these model-based findings. First of all, the model for healthy subjects distinguishes a stable and a fast fraction in the turnover. It would be intuitively attractive to hypothesize that the fast fraction lies more on the outside of the myelin wrapping, and the stable fraction lies more closely to the axon, which is therefore harder to replace[26]. When considering myelin physiology, we expect that the extent of replacement of myelin close to the axon is low in healthy subjects and that the effect of labelling of this stable fraction is therefore very limited. Therefore, this may have only limited effect in the model for turnover in healthy subjects. In patients with progressive MS, this processis altered[27]. As demyelination exposes more of the stable fraction of myelin, we would expect that more of this stable fraction-myelin is broken down, which then contributes to the levels of β-GALC and NO-sulf present in the CSF. This part of myelin will have fewer deuterium-atoms incorporated due to its formation over a longer period of time, starting from before the onset of deuterium labelling, which could explain a lower labelling fraction in the breakdown products.

Secondly, evidence exists that remyelination in MS patients is ineffective[27]. This could slow down the formation of new myelin and thereby decrease the rate of incorporation of deuterium-atoms into myelin molecules. Either of these two pathophysiological processes could explain the lower level of labelling observed in patients with MS.

A third scenario, is that a combination of these processes, namely degradation of stable myelin and ineffective remyelination process, underlies our observations.

Deuterium fraction in body water results showed individual differences, which were comparable to the differences found in the healthy subjects cohort (figure 2). A maximum reported fraction in the MS cohort of 0.037 (table 2) the dosing of 120 mL for 70 days was considered safe[28,29]. From our previous study we know that these fractions should be high enough to label myelin breakdown products [14]. The individual differences in body water turnover seemed to explain most of the interindividual differences seen in the labelling enrichment of β-GALC, as the degree of interindividual variability in the β-GALC kinetics is limited (<10% CV, Table 3).

A limitation of the current study is its small sample size. As this was a proof-of-concept study, we only enrolled 6 healthy subjects and 5 patients completed the study. Exploring the effect of disease duration, and type of MS (primary of secondary progressive MS) would be very interesting but was not feasible due to the small sample size.

The model that describes the kinetics of body water and myelin breakdown products has several limitations. For example, because we had no deuterium enrichment data of intermediate biochemical product, the model directly links body water enrichment with the enrichment of the myelin breakdown products in the CSF with the single turnover rate constant. In this study, we interpreted this turnover rate constant as being representative of the myelin turnover rate, under the assumption that this is most likely the rate-limiting step. Additionally, the data did not allow the estimation of the turnover rate of the stable fraction of myelin, due to a negligible degree of deuterium labelling in this fraction.

In all patients we measured a difference in volume NORPH of lesions between start of the study and after 6 months (table 3). The increase in three patients indicates an increase in the demyelinating process over time during the course of the study. However, we did not find a significant correlation between the kinetics of the myelin breakdown products and the change in volume NORPH of lesions among the 5 patients ($p>0.05$).

CONCLUSIONS

Myelin kinetics of myelin breakdown products in the CSF are significantly different in patients with MS compared to healthy subjects. This can be caused by slower myelin production in these patients, by a higher level of degradation of the stable component of myelin, or, most likely, by a combination of these two processes.

Deuterium labelling in combination with lumbar punctures is a useful method for quantification of metabolic processes of the central nervous system. This method in patients with MS can be used to quantify myelin turnover and can therefore be suitable for the use in proof of concept studies with remyelination compounds in patients with progressive MS.

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TABLE 1 – Subject characteristics (n=5)

Characteristic	Mean (range) or number (%)
Age (years)	54.6 (41–65)
Sex	
Male/female	4 (80) / 1 (20)
Weight (kg)	69.1 (55.3–79.6)
Height (m)	1.77 (1.65–1.84)
BMI	22.1 (18.3–24.1)
Years of MS	17.8 (10-32)

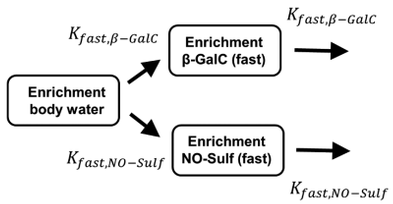
TABLE 2 – Parameters estimates of the precursor-product model describing deuterium enrichment data in body water, β-GALC and NO-sulf.

Parameters	Parameter estimates (RSE%)	
Body water (precursor) parameters		
Water intake (L/day)[1]	3.39 (8.7%)	
Body water pool (L)	38.7 (4.9%)	
Interindividual variability of water intake (CV%)	28.8 (45%)	
Additive residual error	0.00163 (39%)	
Myelin lipid (product) parameters	**β-GALC**	**NO-Sulf**
Fraction with non-negligible turnover in healthy subjects*	0.344 (12%)	0.366 (15%)
Relative fraction with non-negligible turnover in MS patients (compared to healthy subjects) *	0.817 (6.8%)	0.506 (16%)
Turnover rate of non-negligible fraction (day-1)	0.00186 (11%)	0.00714 (9.6)
Inter-individual variability of fraction with fast turnover (CV%)	7.4 (93.1%)	24.3 (32%)
Additive residual error	0.00011 (26%)	0.00044 (44%)

CV = coefficient of variation, RSE = relative standard error, HV = Healthy subjects
1 = For an individual with a total body water of 35.6L predicted with the Watson Formula (24). For all parameters, a single parameter value is estimated for both healthy subjects and MS patients, except the fractions with non-negligible turnover (*)

FIGURE 1 – Schematic overview of the compartmental model describing the kinetics of the myelin breakdown product β-GALC and NO-sulf. The deuterium enrichment of the myelin breakdown products in CSF that originate from the stable faction is assumed to be negligible. $K_{fast,\beta\text{-}galc}$ = turnover rate of the fast fraction of β-GALC, $F_{fast,\beta\text{-}galc}$ = fraction of β-GALC in CSF that originates from the fraction with fast turnover, $K_{fast,NO\text{-}Sulf}$ = turnover rate of the fast fraction of NO-Sulf, $F_{fast,NO\text{-}Sulf}$ = fraction of NO-sulf in CSF that originates from the fraction with fast turnover.

$$Enrichment\ \beta\text{-}GalC\ in\ CSF = Enrichment\ \beta\text{-}GalC\ (fast) \times F_{fast,\beta\text{-}GalC}$$



$$Enrichment\ NO\text{-}Sulf\ in\ CSF = Enrichment\ NO\text{-}Sulf\ (fast) \times F_{fast,NO-Sulf}$$

FIGURE 2 – Deuterium fraction in body water: Observation (dots), population prediction (dark grey dashed line), individual prediction (light grey dashed line). The vertical line at 70 days marks the end of the labeling period. Data from the previously published study in healthy subjects was adapted from the original publication 14) with permission.

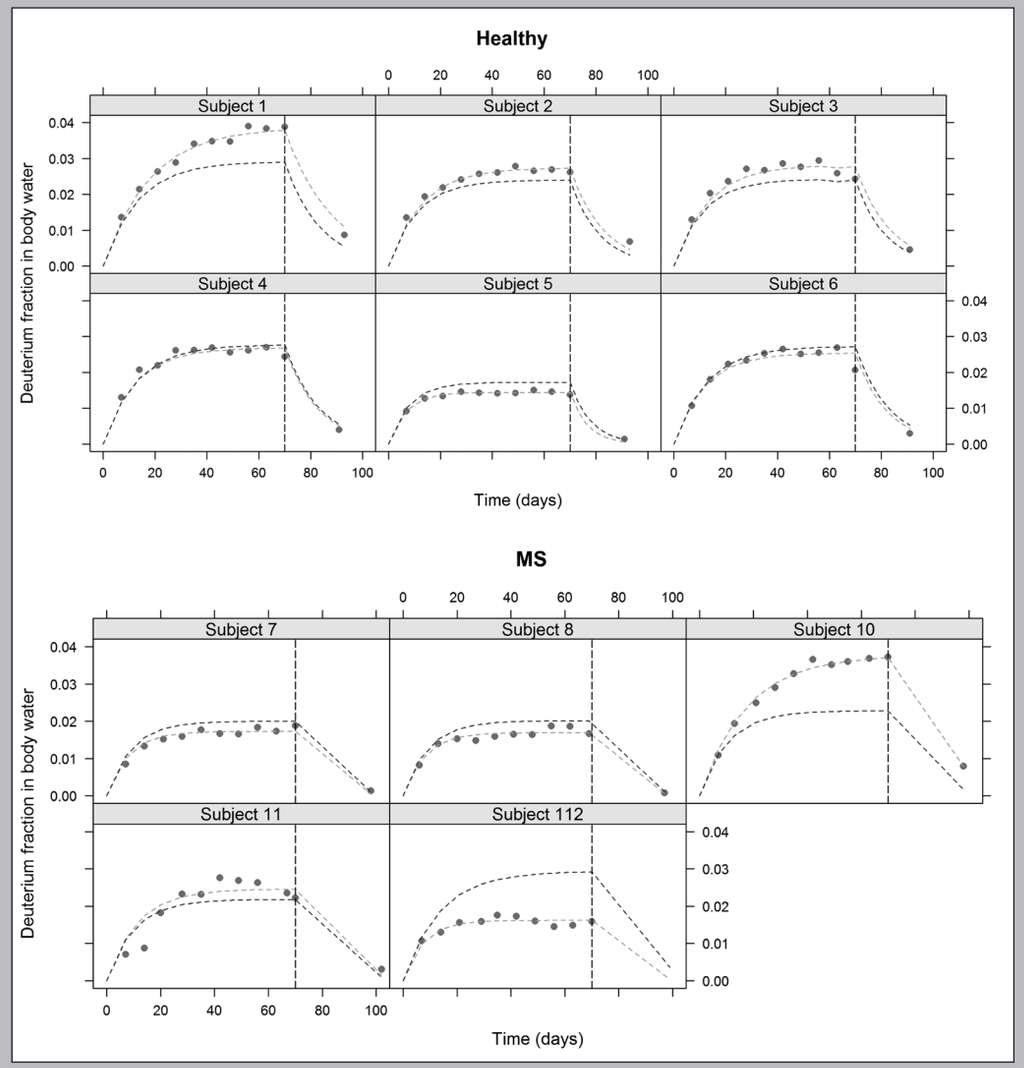


FIGURE 3 – Deuterium fraction in β-GALC: Observation (dots), population prediction (dark grey dashed line), individual prediction (light grey dashed line). The vertical line at 70 days marks the end of the labeling period. Data from the previously published study in healthy subjects was adapted from the original publication 14) with permission.

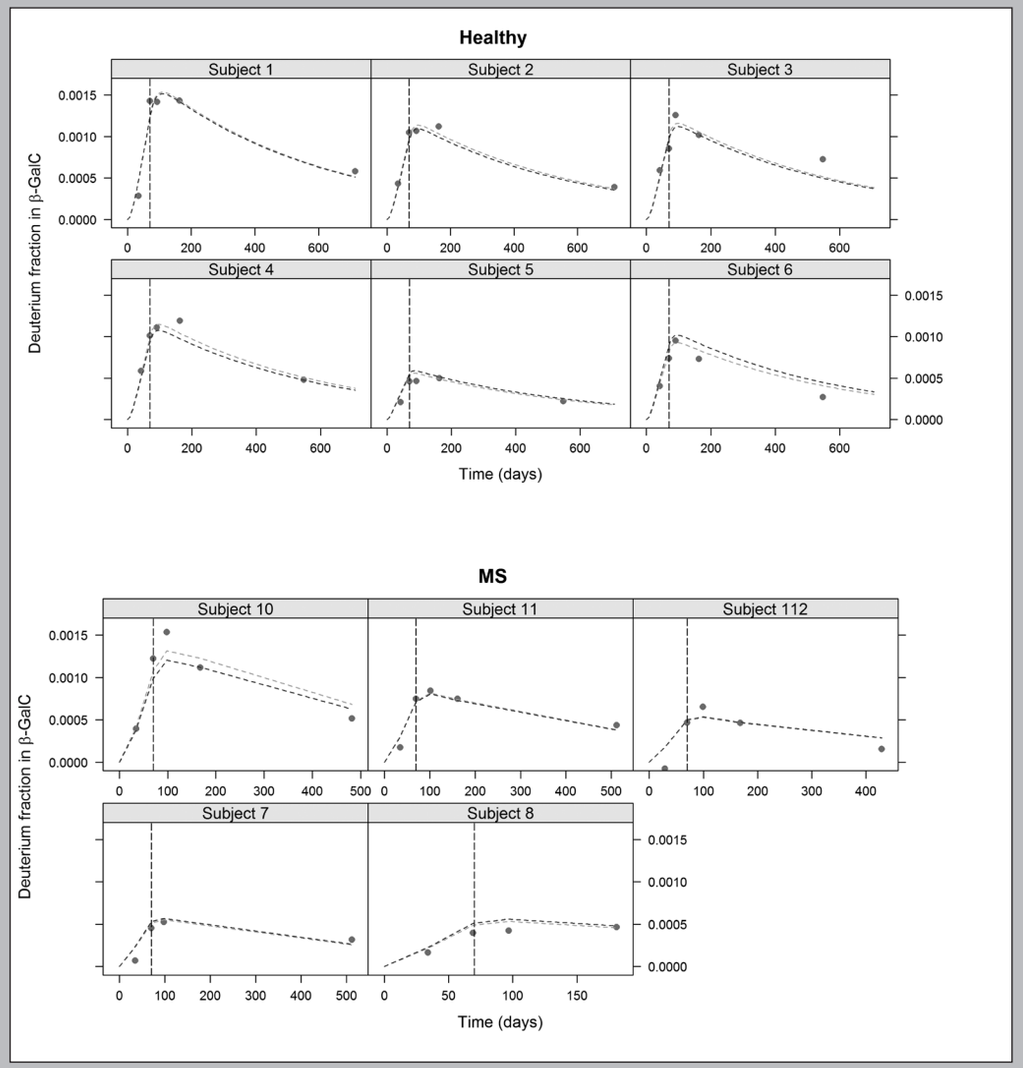


FIGURE 4 – Deuterium fraction in NO-Sulf: Observation (dots), population prediction (dark grey line), individual prediction (light grey line). The vertical line at 70 days marks the end of the labeling period.

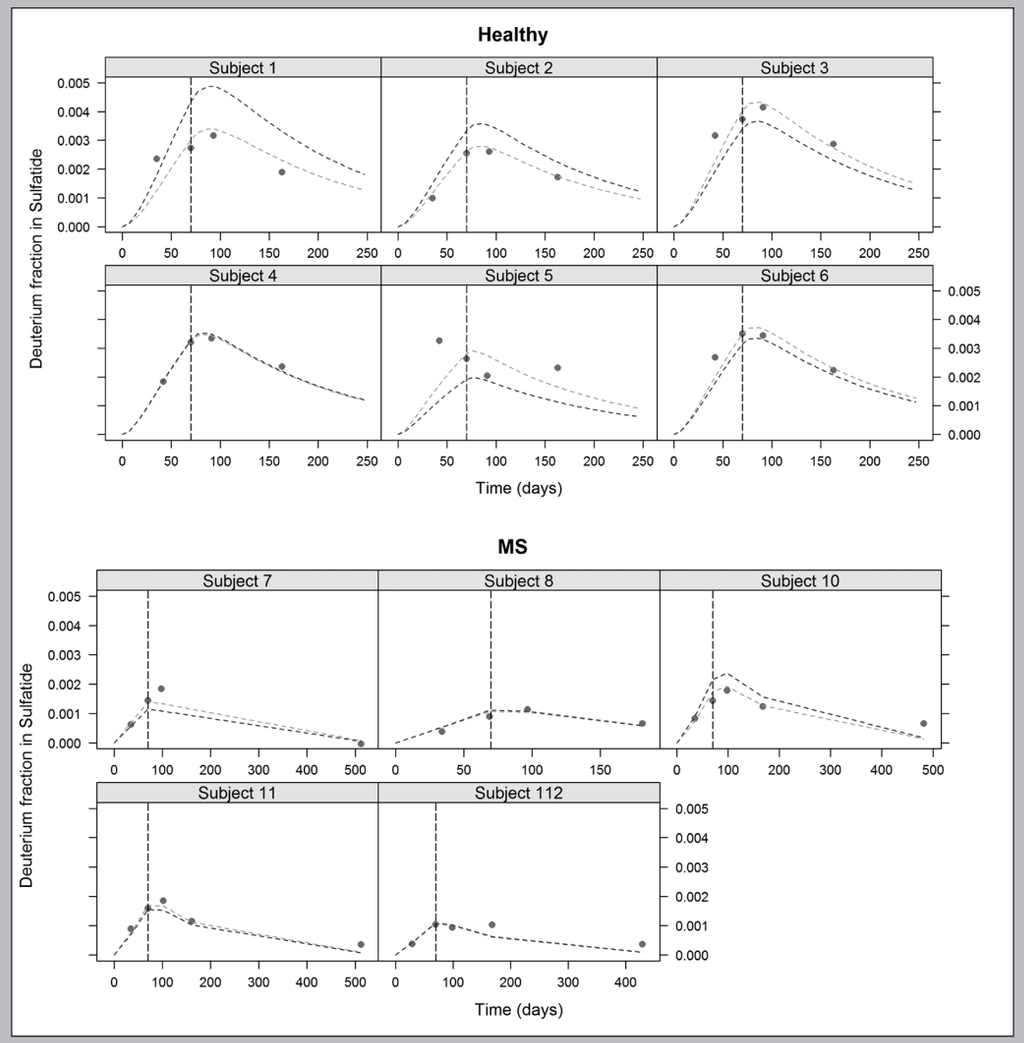


FIGURE 5 – Model predictions for a typical healthy subject and a typical MS patient of deuterium fraction in β-GALC (left) and NO-sulf (right). The simulation is based on the design described in this study: subject receive 120 mL heavy water 70% D_2O) daily, for 70 consecutive days.It shows visually what the effect is of the parameter that estimates the difference between MS patients (grey) and healthy subjects (black) with respect to the fraction of non-negligible turnover.