


ARTICLE

Kinetics of myelin breakdown products: A labeling study in patients with progressive multiple sclerosis

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Abstract

The majority of disease modifying therapies for multiple sclerosis (MS) reduce inflammation, but do not target remyelination. Development of remyelinating therapies will benefit from a method to quantify myelin kinetics in patients with MS. We labeled myelin in vivo with deuterium, and modeled kinetics of myelin breakdown products β -galactosylceramide (β -GalC) and N-Octadecanoyl-sulfatide (NO-Sulf). Five patients with MS received 120 ml 70% D₂O daily for 70 days and were compared with six healthy subjects who previously received the same procedure. Mass spectrometry and compartmental modeling were used to quantify the turnover rate of β -GalC and NO-Sulf in cerebrospinal fluid (CSF). Turnover rate constants of the fractions of β -GalC and NO-Sulf with non-negligible turnover were 0.00186 and 0.00714, respectively, in both healthy subjects and patients with MS. The turnover half-life of β -GalC and NO-Sulf was calculated as 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). Kinetics of myelin breakdown products in the CSF are different in patients with MS compared with healthy subjects. This may 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. Labeling myelin breakdown products is a useful method that 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.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Myelin components can be labeled in healthy subjects using deuterium, allowing us to model the kinetics of the myelin breakdown product β -galactosylceramide and N-Octadecanoyl-sulfatide. This approach offered a method to quantify changes in myelin kinetics after treatment with a remyelinating compound.

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WHAT QUESTION DID THIS STUDY ADDRESS?

Can we measure myelin kinetics in patients with multiple sclerosis (MS), and is there a difference in kinetics of myelin breakdown products between healthy volunteers and patients?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Kinetics of myelin breakdown products are different in patients with MS compared with healthy subjects. The pattern observed between the patients is similar.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Labeling myelin breakdown products can be used to quantify myelin turnover in patients with MS and this method can be used in proof-of-concept studies with remyelination therapies.

INTRODUCTION

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 and at best attenuate neuronal degeneration which will therefore still progress, leading 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 rHlgM22 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 magnetic resonance imaging (MRI), magnetization transfer imaging (MTI), and diffusion-weighted imaging.¹⁰ In addition, non-MRI methods, like positron emission tomography using [11C]PiB,¹¹ or visual evoked potential⁶ can be used. Although these techniques are improving in accuracy, there is still a need to develop more sensitive and specific methods,¹² especially because 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 to obtain a better characterization of the profile of myelin breakdown products. Sulfatide is a sulfated glycosphingolipid found at high concentrations in the myelin sheath. Over 70% of the myelin sheath is comprised of lipids, and around 30% of myelin lipids are sulfatide and its non-sulfated precursor β -GalC.¹⁵ Both lipids can be regarded, when present in the cerebrospinal fluid (CSF), as being specific for central nervous system (CNS) myelin.¹⁶ MRI scans were performed to compare our findings with imaging correlates of demyelination.¹⁷

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 MS. 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 criteria¹⁸ for at least 1 year. Patients had to be between 18 and 70 years old and have a body mass index of 18–30 kg/m² 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 to assess eligibility. Key exclusion criteria were: contraindications for lumbar puncture, contraindication of an MRI, clinically

significant abnormalities during screening and a relapse in 1 month before start of the study.

Study design

During the study, six patients with progressive MS were instructed to consume 120 ml of 70% D₂O daily for 70 days. Weekly urine samples were collected and used to measure the percentage of D₂O 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 to 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 days 0 and 35 (before the first LP) and one on day 167 (fourth LP), to compare changes in imaging biomarkers related to myelination to the deuterium labeling results.

Published and unpublished data from our previous study in healthy subjects¹⁴ were used not only to quantify the kinetics of myelin breakdown in patients, but also to compare them to healthy subjects through simultaneous modeling. The methodology used in the current study was exactly identical to the methodology used in the previous study in healthy subjects.¹⁴

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.^{14,19,20} D₂O 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 ¹³C₃ acetone is then determined using gas chromatography/mass spectroscopy analysis. The deuterium fraction in the urine sample was calculated using the ratio of singly deuterated to nondeuterated forms. Measuring D₂O in urine has been established as reliable marker of body water D₂O, and has been used in other studies.^{21–23} 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. NO-Sulf results from the healthy subject cohort were not reported in the previous study but are reported in the current report.

Compartmental model

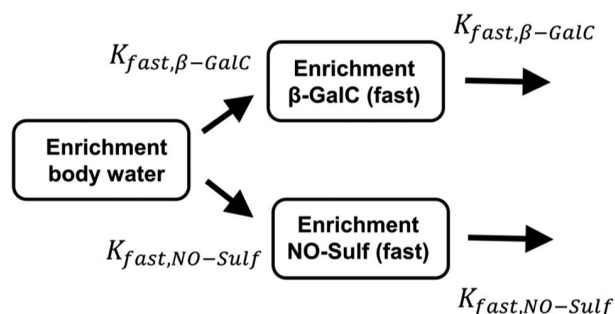
A compartmental model that describes the turnover of body water (precursor) and the myelin lipid (product) was fitted to the enrichment data of β -GalC and NO-Sulf using the nonlinear mixed effects modeling program NONMEM, version 7.3.0.²⁴ The first-order conditional estimation with interaction method was used to estimate typical parameter values and their interindividual 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.²⁵ 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.

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



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

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 fraction is assumed to be negligible. $K_{\text{fast},\beta\text{-GalC}}$ = turnover rate of the fast fraction of β -GalC, $F_{\text{fast},\beta\text{-GalC}}$ = fraction of β -GalC in CSF that originates from the fraction with fast turnover, $K_{\text{fast},\text{NO-Sulf}}$ = turnover rate of the fast fraction of NO-Sulf, $F_{\text{fast},\text{NO-Sulf}}$ = fraction of NO-Sulf in CSF that originates from the fraction with fast turnover. β -GalC, β -galactosylceramide; CSF, cerebrospinal fluid; NO-Sulf, N-Octadecanoyl-sulfatide

Magnetic Resonance Imaging

To study changes in tissue integrity in the normal appearing white matter, high resolution structural MRI (3D-T1 and fluid-attenuated inversion recovery [FLAIR]) and MTI scans were performed. The 3D-T1-weighted scans were made to segment white matter from CSF and from gray matter. FLAIR images were used to segment white matter lesions from the white matter. The MTI-ratio was calculated in the remaining normal appearing white matter and lesions separately.²⁶ MRI was performed before the first LP, and a second one 167 days after the 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: 3D-T1-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 min. FLAIR: TR = 11,000 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 scans: 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 dropouts

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, kg/m ²	22.1 (18.3–24.1)
Years of MS, years	17.8 (10–32)

Abbreviations: BMI, body mass index; MS, multiple sclerosis.

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. MS-related medication reported was Baclofen (subjects 7, 8, and 10), tizanidine (subject 8), Teriflunomide (subject 112), and Fampridine (subject 112). Missing data in the analysis are the LP four for subject seven (LP failed) and LP five for subject eight (subject did not participate). Most of the reported adverse events were mild and transient. One serious adverse event was reported: subject eight was admitted to the hospital 4 months after the 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 D₂O. 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 study¹⁴ reached steady-state deuterium enrichment in body water during the 70-day labeling period (see Table 2 and Figure 2). There was considerable interindividual 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 D₂O 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 two to three-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 patients with MS (see Table 2 and Figure 4).

Compartmental model output

Interindividual 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 (Figures 2–4).

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

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 ^a	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 with healthy subjects)*	0.817 (6.8%)	0.506 (16%)
Turnover rate of non-negligible fraction (day ⁻¹)	0.00186 (11%)	0.00714 (9.6)
Interindividual variability of fraction with fast turnover (CV%)	7.4 (93.1%)	24.3 (32%)
Additive residual error	0.00011 (26%)	0.00044 (44%)

Abbreviations: β-GalC, β-galactosylceramide; CV, coefficient of variation; MS, multiple sclerosis; NO-Sulf, N-Octadecanoyl-sulfatide; RSE, relative standard error.

^aFor an individual with a total body water of 35.6 L predicted with the Watson Formula.²⁴ For all parameters, a single parameter value is estimated for both healthy subjects and patients with MS, except the fractions with non-negligible turnover (*).

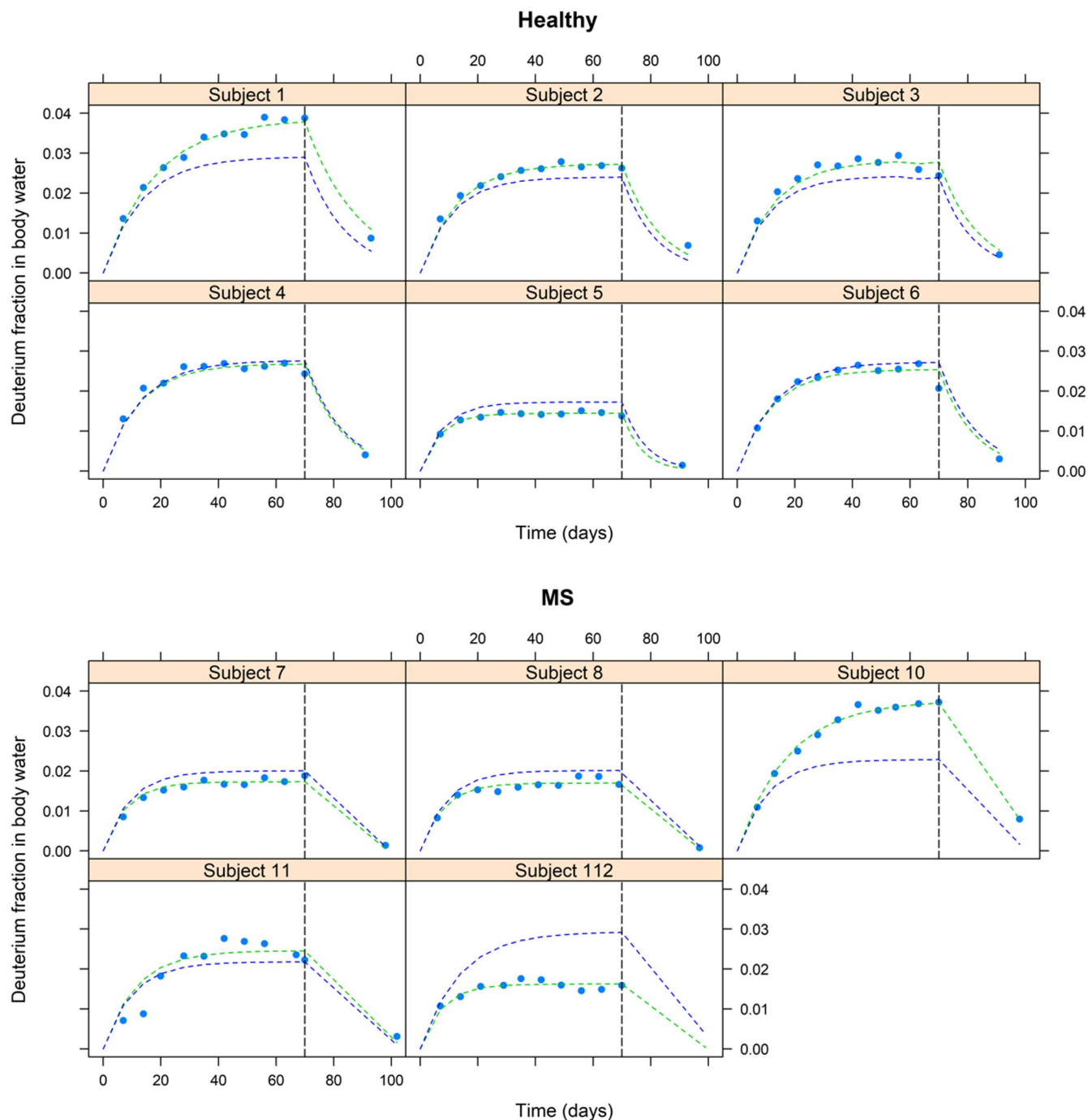


FIGURE 2 Deuterium fraction in body water. Observation (blue dots), population prediction (blue line), individual prediction (green line). The vertical line at 70 days marks the end of the labeling period. Data from the previously published study in healthy subjects was adapted from the original publication¹⁴ with permission. MS, multiple sclerosis

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 (coefficient of variation [CV] = 24.3%).

We observed a significant difference in the turnover of both myelin lipids between the healthy subjects and the patients with MS: 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 a model where the effect was included

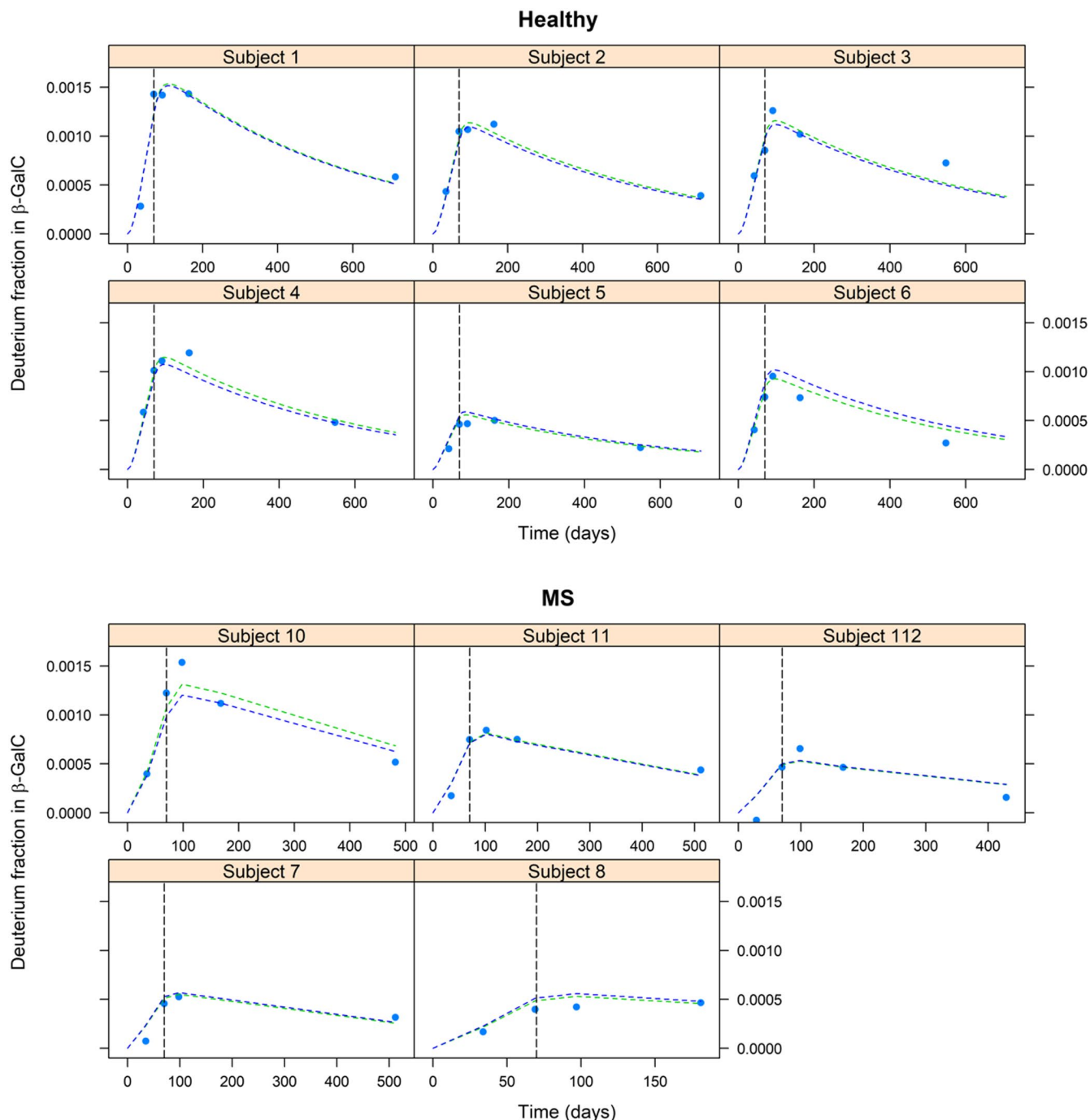


FIGURE 3 Deuterium fraction in β -GalC. Observation (blue dots), population prediction (blue line), individual prediction (green line). The vertical line at 70 days marks the end of the labeling period. Data from the previously published study in healthy subjects was adapted from the original publication¹⁴ with permission. β -GalC, β -galactosylceramide; MS, multiple sclerosis

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 patient with MS 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).

We found no significant difference between the turnover of body water of the patients with MS and that of healthy volunteers.

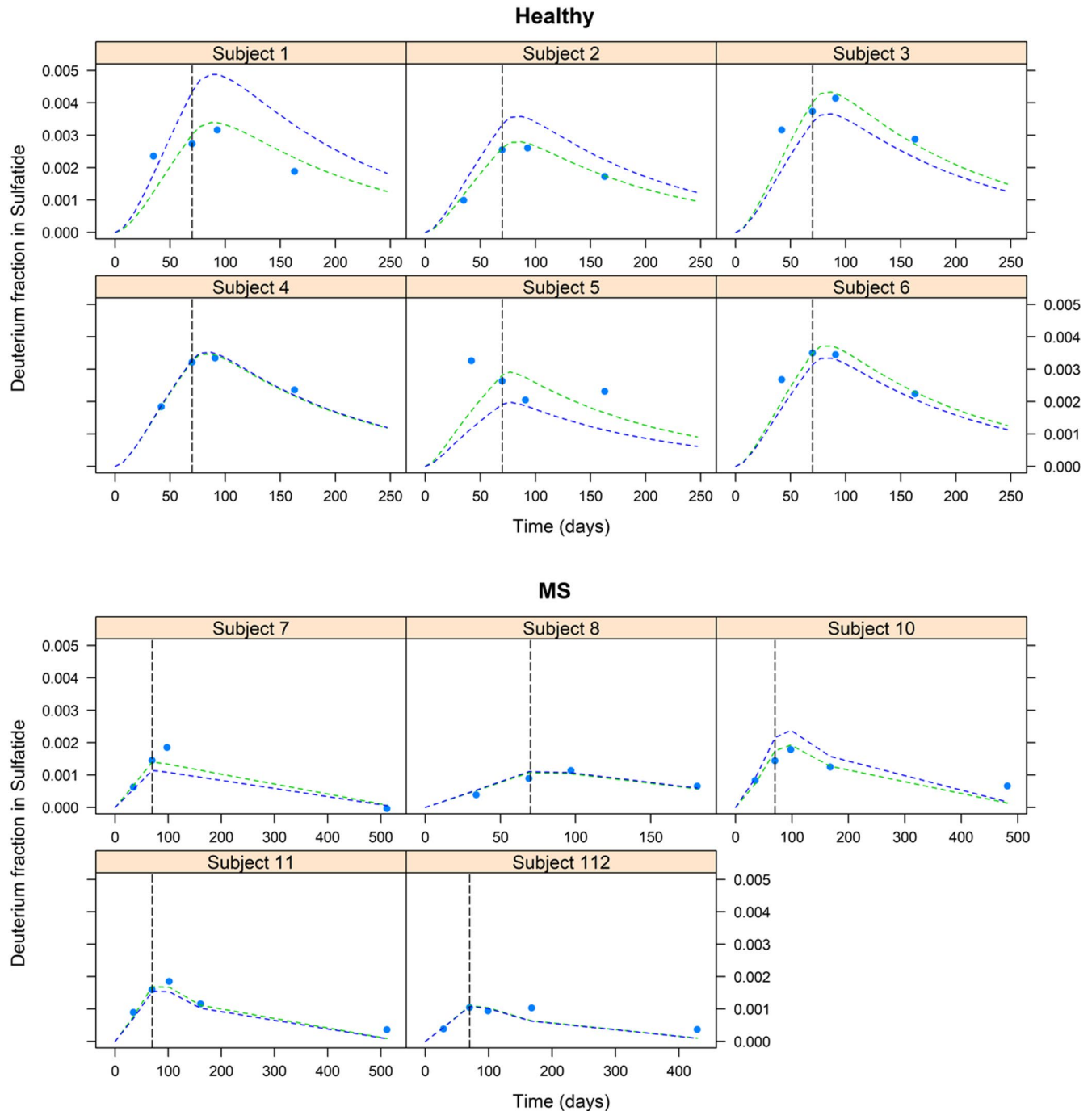


FIGURE 4 Deuterium fraction in NO-Sulf. Observation (blue dots), population prediction (blue line), individual prediction (green line). The vertical line at 70 days marks the end of the labeling period. MS, multiple sclerosis; NO-Sulf, N-Octadecanoyl-sulfatide

Magnetic Resonance Imaging

The normalized peak heights (norPHs) of the normal appearing white matter and of the white matter hyperintensity lesions were calculated for all patients at both time points. The MTI norPH in the normal appearing white matter decreased in four 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 a decrease in norPH of the lesions (−11.7 and −14.4%; Table S1).

Discussion

This is the first study to measure the kinetics of deuterium-labeled myelin breakdown products β -GalC and NO-Sulf in

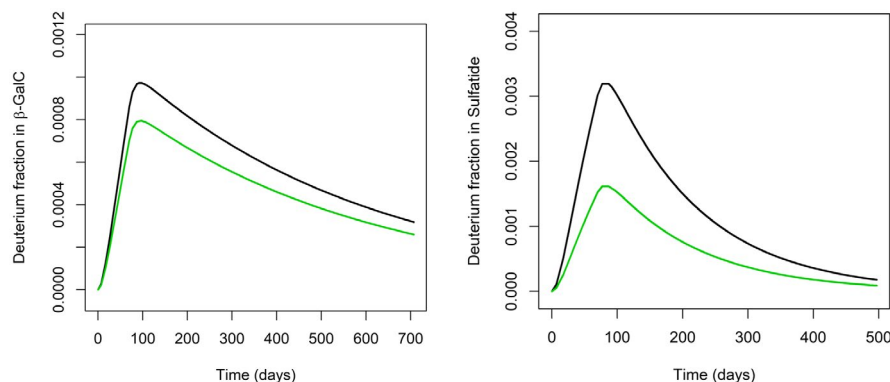


FIGURE 5 Model predictions for a typical healthy subject and a typical patient with MS of deuterium fraction in β -GalC (left) and NO-Sulf (right). The simulation is based on the design described in this study: subject receive 120 ml heavy water (70% D_2O) daily, for 70 consecutive days. It shows visually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

the CSF of patients with progressive MS. In a pooled compartmental analysis, we analyzed 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 patients with MS than in healthy subjects, which indicates that myelin formation was slower, or breakdown of a more stable myelin component was higher. As formation of the more stable myelin component occurs at lower speed, deuterium incorporation will be lower. If the relative contribution to CSF breakdown products of this component was higher, it may have led to dilution and therefore lower levels of labeling.

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 patients with MS compared with the healthy subjects: 49.9% lower for NO-Sulf, and 18.3% lower for β -GalC. In the model, there is no difference in the turnover rate of the non-negligible fraction between patients with MS and healthy subjects. There are several possible physiological explanations for these model-based findings. First of all, the model 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.²⁷ 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 labeling 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 process is altered.²⁸ 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 labeling, which could explain a lower labeling fraction in the breakdown products.

Second, evidence exists that remyelination in patients with MS is ineffective.²⁸ 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 labeling 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.^{29,30} 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 labeling enrichment of β -GalC, as the degree of interindividual variability in the β -GalC kinetics is limited (<10% CV; Table 2).

A limitation of the current study is its small sample size. As this was a proof-of-concept study, we only enrolled six healthy subjects and five patients completed the study. Exploring the effect of disease duration, and type of MS (primary or secondary progressive MS) would be very interesting but was not feasible due to the small sample size. Among patients with secondary progressive MS, sometimes a more subtle relapsing and remitting pattern can be distinguished,³¹ whereas in primary progressive

MS, a more stable level of continuous demyelination (and remyelination) could be expected. How these may have influenced the labeling level and estimated turnover of breakdown products in the CSF is unclear, however, it could be expected to be more variable among patients with secondary progressive MS, as recent bouts of demyelination will likely influence the formation of new myelin.

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 labeling in this fraction. In all patients, on repeated MRI scans, we measured a difference in volume norPH of lesions between start of the study and after 6 months (Table S1). 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 five patients ($p > 0.05$).

A disadvantage of the current method is the need to perform repeated LPs. We have considered to measure β -GalC and NO-Sulf in the peripheral circulation, however, as these are also present in the pancreas,³² peripheral levels do not necessarily represent the levels induced by demyelination in the brain. We have recently found that 24S-hydroxy-cholesterol is measurable in the peripheral circulation and may also reflect demyelination in the brain.³³⁻³⁵ We are currently looking into this method, which may provide a reliable alternative to CSF sampling, and would represent a less invasive method for patients.

Serum neurofilament light (sNfL) is a plausible marker of neurodegeneration that can be measured accurately, sensitively, and reproducibly.³⁶ Although this study focused on myelin breakdown, sNfL might be an interesting avenue for further research.

Myelin kinetics of myelin breakdown products in the CSF are significantly 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 the stable component of myelin, or, most likely, by a combination of these two processes.

Deuterium labeling in combination with LPs is a useful method for quantification of metabolic processes of the CNS. 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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CONFLICT OF INTEREST

A.V., G.D., and J.C. are (previous) employees of Biogen. All other authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

K.M.S.K., S.C.G., J.v.d.G., A.C.H., A.V., H.M., and G.J.G. wrote the manuscript. K.M.S.K., S.C.G., J.v.d.G., T.H., A.V., J.C., G.D., and G.J.G. designed the research. K.M.S.K., S.C.G., J.v.d.G., A.C.H., H.M., and G.J.G. performed the research. S.C.G. and J.v.d.G. analyzed the data.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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