Normal-appearing White Matter in Patients with Phenylketonuria: Water Content, Myelin Water Fraction, and Metabolite Concentrations[1]

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Purpose: To prospectively assess relative water content (RWC), myelin water fraction (MWF), and hydrogen 1 magnetic resonance (MR) spectroscopy findings in the white matter (WM) of patients with phenylketonuria (PKU).

Materials and Methods: This study was approved by the institution's investigational review board, and informed consent was obtained. T2 water relaxation data were acquired by using a 48-echo measurement in a transverse plane through the genu and splenium of the corpus callosum in 16 patients (six men, 10 women; age range, 18–40 years) with PKU and 16 age- and sex-matched control subjects. MR spectroscopy was performed in a voxel (94 × 70 × 15 mm) above the ventricles. WM in control subjects (defined as normal WM) was compared with normal-appearing WM (NAWM) and diffuse WM lesions in patients with PKU by using a Student t test.

Results: Patients with PKU had two forms of NAWM: *(a)* areas that looked normal on intermediate-weighted (IW) and T2-weighted MR images and long T2 maps and *(b)* areas that looked normal on IW and T2-weighted MR images but were hyperintense on long T2 maps. Both forms of NAWM showed increased RWC (up to 2.5%, $P < .001$) and reduced MWF (up to 56%, $P < .001$) relative to normal WM; these changes paralleled those seen in diffuse WM lesions. Approximately 9% of the water in diffuse WM lesions was in a reservoir with a long T2 time of 200–800 msec. Myoinositol concentrations were reduced by 14% ($P = .003$) in patients with PKU.

Conclusion: In patients with PKU, NAWM and diffuse WM lesions have altered RWC and MWF relative to normal WM, and diffuse WM lesions show a redistribution of water into an extracellular reservoir with a long T2 time.

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Phenylketonuria (PKU) is an inborn error of phenylalanine metabolism that causes severe mental retardation in most affected individuals who are not treated with a diet restricted in phenylalanine. Some affected individuals, however, do not develop progressive brain damage despite high blood levels of phenylalanine, and this may, in part, be related to reduced phenylalanine transport into the brain (1,2). Given that long-term dietary compliance is difficult and costly in patients with PKU, it would be useful in clinical management to identify patients at risk of long-term neurologic sequelae from their disease.

White matter (WM) abnormalities, which are symmetric and concentrated in the posterior periventricular regions, are frequently seen on conventional magnetic resonance (MR) images but do not correlate with the patient's clinical status (3). Metabolite analysis by using hydrogen 1 (^{1}H) MR spectroscopy does not always help distinguish between patients with PKU and control subjects (4,5). Measurement of brain phenylalanine levels by using MR spectroscopy shows promise, both to determine patients who may be at risk of sequelae (5) and to evaluate therapeutic approaches (6). Unfortunately, there are methodologic concerns with measuring brain phenylalanine (7), and not all authors have been able to identify the brain phenylalanine peak (8) or to correlate measurements of brain phenylalanine with the cognitive status of the patients (9).

MR T2 relaxation measurements in WM have the potential to provide more specific information about the progressive brain damage that may occur in PKU. The proton MR signal from normal brain arises almost entirely from water. Results of previous T2 relaxation studies in human brains (10) have shown that the water signal can be separated into three components: a very long T2 component (approximately 2 seconds) from cerebrospinal fluid, an intermediate component (approximately 100 msec) arising from intra- and extracellular water, and a short T2 component (approximately 20 msec) attributed to water trapped between the myelin bilayers (known as "myelin water"). The relative water content (RWC) of a tissue is calculated as the ratio of the total signal to some known standard. The ratio of the myelin water signal (short T2 component) to the total signal gives the myelin water fraction (MWF). Findings of histologic studies have shown that the MWF qualitatively and quantitatively corresponds to the anatomic distribution of myelin (11,12). In multiple sclerosis, alterations in brain RWC and MWF have been demonstrated in normal-appearing WM (NAWM) (13), and evaluation of changes in NAWM has been shown to be a sensitive indicator of disease activity (14).

The purpose of our study was to prospectively assess RWC, MWF, and ^{1}H MR spectroscopy findings in the WM of patients with PKU.

Materials and Methods

Patient Information

This study was approved by the investigational review board of our institution (University of British Columbia) and was supported solely by a Vancouver Hospital and Health Sciences Centre Interdisciplinary Grant. The authors had control of all data and information submitted for publication. All subjects with PKU who attended our clinic between October 2000 and November 2002 and who were able to provide informed consent were invited to participate. Written informed consent was obtained from patients with PKU and from control subjects. All patients with PKU had been prescribed a low-phenylalanine diet from birth and had been maintained on the diet for at least 2 years prior to the study, although compliance with this diet was highly variable. All patients with PKU were able to live independently and were considered cognitively normal. Control subjects, who were matched for age and sex (Table 1) and were interviewed to ensure that they had no history of neurologic disease or PKU, were recruited between November 2002 and August 2003 by using posted advertisements in our hospital. Phenylalanine blood levels were measured immediately before and after the MR examination by using standard blood dot techniques. A total of 16 patients and 16 control subjects (Table 1) participated in the study. However, not all data were available on all subjects because some data were lost due to file corruption or motion artifact. The number of subjects for whom data are available is defined in the article for each measurement reported.

MR Imaging

MR imaging was performed with a 1.5-T system (Echo Speed; GE Medical Systems, Milwaukee, Wis) operating on the version 5.7 platform. Localizer, intermediate-weighted (IW), and T2-weighted images (repetition time msec/echo time msec, 2500/30 and 80) were

Advances in Knowledge

- In patients with phenylketonuria, white matter that appears normal on conventional MR images is not normal but demonstrates increases in relative water content and reductions in myelin water fraction.
- Some areas of the brain in patients with phenylketonuria have a water reservoir with a prolonged T2 relaxation time.

followed by a 48-echo modified Carr-Purcell-Meiboom-Gill sequence with variable repetition time (15). For the Carr-Purcell-Meiboom-Gill T2 relaxation measurement, a single transverse section (Fig 1a) was acquired through the base of the genu and splenium of the corpus callosum (5 mm thick; 128 × 128 matrix; echo time, 10 msec for the first 32 echoes and 50 msec for the last 16 echoes; and four signals acquired). For the 20 central lines of k-space, the repetition time was 3.8 seconds and was ramped linearly to 2.12 seconds at the extremities of k-space. The reduction in repetition time had a negligible effect on the estimated T2 distributions but decreased the acquisition time substantially (15).

T2 distributions of the water signal were numerically calculated with a regularized nonnegative least-squares algorithm (16). The RWC was calculated as the ratio of the signal (extrapolated to an echo time of 0 msec) from the chosen region of interest (Fig 1) to that from an internal gray matter standard (the putamen), because the putamen has minimal contamination from cerebrospinal fluid (13). It was assumed that the putamen water contents would be similar for patients with PKU and control subjects. Results of a previous study in patients with multiple sclerosis showed that the putamen T1 time, which is related to its water content (17), was similar in patients and control subjects (18). The MWF was calculated by integrating the T2 distribution between 10 and 50 msec and dividing it by the T2 distribution integrated over the entire T2 range (10 msec to 5 seconds). Similar to the MWF, a "long T2 component" fraction was calculated by integrating the T2 distribution between 200 and 800 msec and dividing it by the T2 distribution integrated over the entire range. For each subject, a long T2 map was generated for the transverse section. The relationship between changes in MWF and total water content was evaluated by using a model developed for multiple sclerosis, which was reported previously (13).

The B_1 field was assumed to be relatively homogeneous across the selected

section because we acquired uniform profiles across similar volumes in oil phantoms, and previous measurements by using external water phantoms gave reasonable water content values throughout this brain level (10). In a previous study of in vivo T1 relaxation (19), in which we used the same head coil, we found that the inversion pulses were remarkably uniform across most of the brain.

Single-voxel ^{1}H MR spectroscopy was performed in all subjects. A large voxel was placed superior to and excluding the lateral ventricles (Fig 1a). The voxel covered as much of the chosen section as possible. The dimensions were 94 × 70 × 15 mm (with standard

deviations of 7, 8, and 0, respectively). Spectra were acquired with a point-resolved spectroscopy sequence (echo time, 30 msec; repetition time, 5 seconds; 2000 Hz; and 2048 complex points). Spectral areas for N-acetylaspartate, choline, creatine, and myoinositol were measured by using LC-Model (version 6.1-0; Stephen Provencher, Oakville, Ontario, Canada) (20), which included a fit to macromolecules, and T1 (21) and T2 (19) corrections were applied.

Metabolite concentrations (22) were found by using the following equation: $(SI_{met}/SI_{wtr}) \cdot (np_{wtr}/np_{met}) \cdot WC \cdot 55\,000$ mmol/L, where SI_{met} and SI_{wtr} are relaxation-corrected metabolite and wa-

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Table 1

Demographic Information and Phenylalanine Levels in Patients with PKU and Control Subjects

Parameter	Patients ($n = 16$)	Control Subjects ($n = 16$)	P Value
Mean age (y) ± standard error	26.6 ± 1.8 (18–40)	27.2 ± 1.6 (19–39)	.77
Male/female ratio	6:10	6:10	NA
Mean blood phenylalanine level (μmol/L)	862 (152–1594)	NA	NA

Note.—Data in parentheses are the range. NA = not applicable.

Figure 1
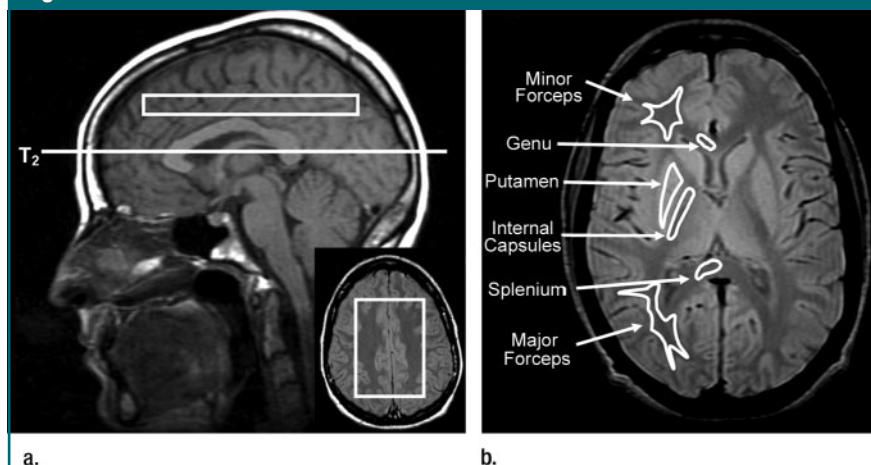

a.   **b.**

Figure 1: **(a)** Sagittal MR image (300/14) shows T2 measurement section (line) and spectroscopy voxel (box). Transverse image (inset; 2200/28) shows anteroposterior and right-to-left extent of voxel. **(b)** Regions of interest of WM structures on IW MR image (2200/28) for analysis of MWF and RWC. Regions of interest were analyzed on both right and left sides.

ter signal intensities, respectively; np_{met} and np_{wtr} are the numbers of protons contributing to the metabolite signal, respectively; WC is the voxel water content; and 55 000 mmol/L is the concentration of water in the voxel if the WC were unity. Images of the voxel were segmented for cerebrospinal fluid (f_{csf}), gray matter (f_{gm}), and WM (f_{wm}) by using Image Pro Plus 4.5 (Media Cybernetics, Silver Spring, Md). The WC was calculated as follows: WC = $WC_{gm} \cdot f_{gm}$ + $WC_{wm} \cdot f_{wm}$ + $1 \cdot f_{csf}$, by using the literature values of 0.83 g/mL for WC of gray matter (WC_{gm}) and 0.71 g/mL for WC of WM (WC_{wm}) (10). The T1 correction to SI_{wtr} took into account the small cerebrospinal fluid contribution (23). The relaxation-corrected water signal was multiplied by $1 - f_{csf}$ so that it was a measure of intra- and extracellular and myelin water only.

Regions of Interest, Lesion, and NAWM Identification

A single author (C.L.) with 8 years of experience in evaluating MR images identified regions of interest in the following structures: the major and minor forceps, internal capsules, genu, and splenium (Fig 1b). This same author classified the WM within these structures into three classes: *(a)* IW-T2-long

T2 NAWM, which indicated WM regions in patients with PKU that appeared normal on the IW and T2-weighted MR images and long T2 maps; *(b)* IW-T2 NAWM, which indicated WM regions that showed normal signal intensity on IW and T2-weighted images but appeared hyperintense on the long T2 maps; or *(c)* diffuse WM lesions, which indicated WM regions that appeared hyperintense on T2-weighted images, which are similar to the definition of "dirty" WM lesions in multiple sclerosis (24). Diffuse WM lesions appeared normal on IW images but hyperintense on the long T2 maps.

WM regions in the control subjects were labeled as normal WM.

Statistical Analysis

Statistical analysis was performed by using a two-tailed Student t test, with $P < .05$ considered to indicate a significant difference, by using Excel 2003 (Microsoft, Seattle, Wash). All errors are expressed as standard errors.

Results

Demographic information on study subjects is shown in Table 1. Diffuse WM lesions were found in 13 patients with PKU, and these lesions were distributed

symmetrically around the midline. Diffuse WM lesions and IW-T2 NAWM were found only in the major and minor forceps. IW-T2–long T2 NAWM was also present in the major and minor forceps. In other structures, all WM was found to be IW-T2–long T2 NAWM.

RWC Findings

The RWC of IW-T2–long T2 NAWM for all the WM structures studied was, on average, 2.9% ($P < .001$; range, 1.9%–3.9% [$P < .003$]) higher than that of normal WM (Fig 2, Table 2), and the increase was significant for all structures. The RWC of diffuse WM lesions, found in the minor and major forceps, was 2.8% ($P = .003$) and 2.5% ($P = .029$), respectively, higher than that of normal WM. The RWC of IW-T2 NAWM was intermediate between that of IW-T2–long T2 NAWM ($P = .14$) and normal WM ($P = .097$), but these differences did not reach statistical significance. The characteristics of diffuse WM lesions, found in the major and minor forceps, are compared with IW-T2 NAWM, IW-T2–long T2 NAWM, and normal WM in Table 2.

MWF Findings

MWF was reduced (Fig 3, Table 2) in IW-T2–long T2 NAWM by an average of 14.0% ($P = .02$; range, 0.6% to

Figures 2, 3

Figure 2: RWC values and standard errors according to structure for normal WM *(NWM)* in 15 control subjects and NAWM and diffuse WM *(DiffWM)* lesions in 15 patients with PKU. Note the increased RWC exhibited in all IW-T2–long T2 NAWM and IW-T2 NAWM structures. *IC* = internal capsules, *Major* = major forceps, *Minor* = minor forceps.
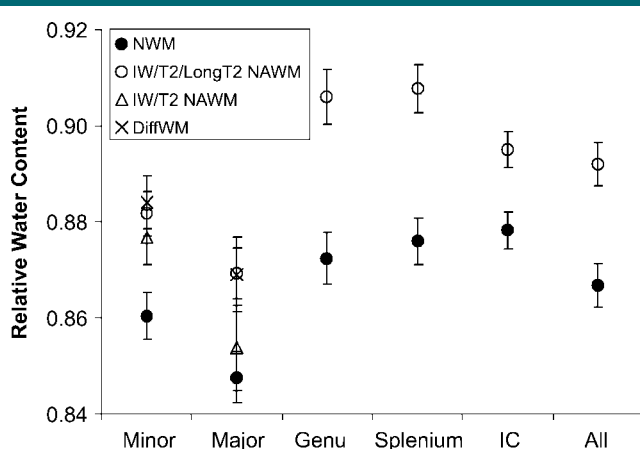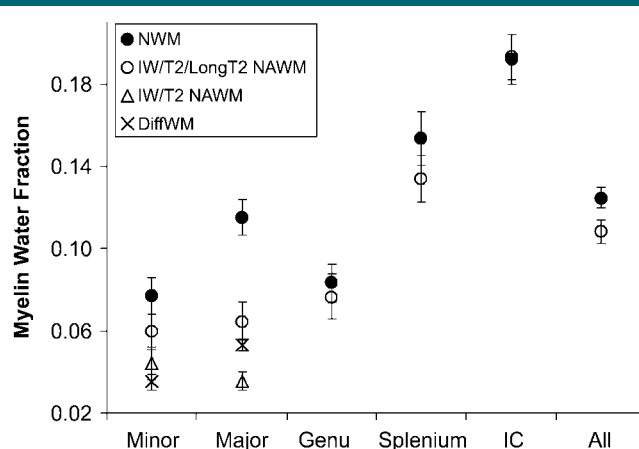
Figure 3: MWF values and standard errors according to structure in normal WM *(NWM)* in 15 control subjects and NAWM and diffuse WM *(DiffWM)* lesions in 15 patients with PKU. Note the decreased MWF in diffuse WM lesions and, on average, all WM. *IC* = internal capsules, *Major* = major forceps, *Minor* = minor forceps.

−43.7%). MWF in diffuse WM lesions in the minor and major forceps of patients with PKU was reduced by an average of 54.0% (−54.3% and −53.8%, respectively; $P < .001$ in both structures) relative to that in normal WM and was reduced by an average of 29.5% (−41.1% [$P = .02$] and −17.9% [$P = .24$], respectively) relative to that in IW-T2–long T2 NAWM. Of interest, the MWF of IW-T2 NAWM was 35.5% lower than that of IW-T2–long T2 NAWM (Table 2) ($P = .006$). We wondered if the observed decrease in MWF was simply because of an increase in total water content. We applied a model developed previously (13) and found that a 4.1% increase in water content would be required to reduce the MWF by the observed 14%. Our observed average increase in RWC was only 2.9%. Furthermore, the required 4.1% increase in RWC would lead to a 12% increase in overall brain volume, which makes this hypothesis unfeasible (13).

Prolonged T2 Peak in Diffuse WM Lesions Suggests Separate Water Reservoir

A T2 peak was identified at approximately 400 msec in the diffuse WM lesion T2 distribution (Fig 4, E), which contained an average of 9% of the water in the region of interest (7.8% and 10.5% for diffuse WM lesions in the minor and major forceps, respectively) and was clearly distinguishable from the main T2 peak at approximately 90 msec and from the cerebrospinal fluid peak (>2 seconds). This peak had a T2 time between 200 and 800 msec and was identified in the WM of 12 of the 15 patients with PKU. The three patients without this peak had no identifiable WM lesions in the section from which the data were acquired.

MR Spectroscopy

There was no difference in absolute concentrations of choline or creatine between control subjects and patients with PKU (Table 3). An increase in *N*-acetylaspartate concentrations in patients with PKU borders on significance ($P = .047$). Patients with PKU had a 14% reduction in myoinositol concentration ($P = .003$) relative to that in

Table 2

RWC and MWF Values in WM and Comparisons between Classes of WM in Major and Minor Forceps in 15 Patients with PKU and 15 Control Subjects

Parameter	RWC	MWF
Mean value ± standard error		
Normal WM	0.854 ± 0.0051	0.096 ± 0.0087
IW-T2–long T2 NAWM	0.876 ± 0.0049	0.062 ± 0.0088
IW-T2 NAWM	0.865 ± 0.0074	0.040 ± 0.006
Diffuse WM lesions	0.877 ± 0.0067	0.044 ± 0.003
P value for comparisons		
IW-T2–long T2 NAWM vs normal WM	<.001 (2.5)	<.001 (−31.1)
IW-T2 NAWM vs normal WM	.097 (1.3)	<.001 (−55.8)
Diffuse WM lesions vs normal WM	<.001 (2.6)	<.001 (−54.0)
Diffuse WM lesions vs IW-T2–long T2 NAWM	.865 (0.1)	.015 (−29.5)
Diffuse WM lesions vs IW-T2 NAWM	.139 (1.3)	.47 (14.4)
IW-T2 NAWM vs IW-T2–long T2 NAWM	.135 (−1.2)	<.001 (−35.5)

Note.—Data in parentheses are the percentage magnitude of difference between the two groups being compared. Only 13 patients with PKU had diffuse WM lesion changes. RWC is increased and MWF is reduced in both IW-T2–long T2 NAWM and IW-T2 NAWM, and these changes parallel those seen in diffuse WM lesions.
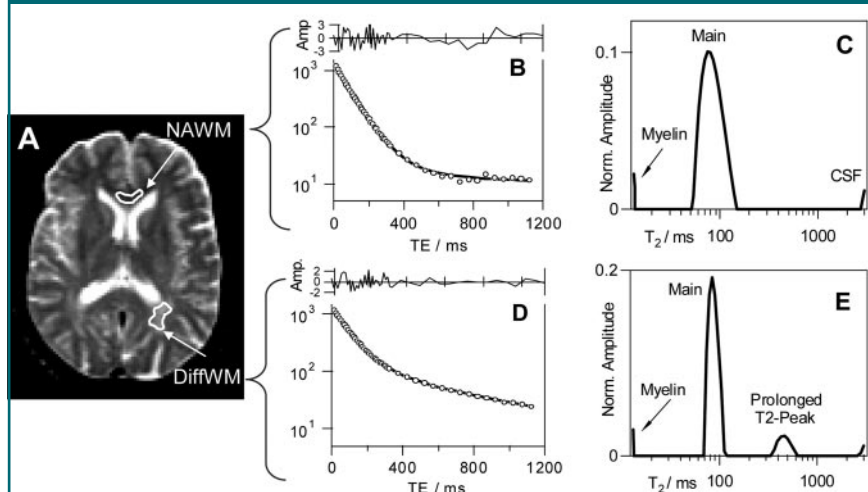
Figure 4

Figure 4: *A*, MR image (3000/90) shows region of interest in patient with PKU for IW-T2–long T2 NAWM and diffuse WM *(DiffWM)* lesions. Panels *B* and *C* show NAWM T2 decay curve and corresponding T2 distribution, respectively. *D* and *E* show diffuse WM lesion T2 decay curve and corresponding T2 distribution, respectively. The residuals of the decay curve fits are shown above the curves in *B* and *D*. Note the prolonged T2 peak in *E*.

control subjects. Segmentation of the voxels showed that cerebrospinal fluid, gray matter, and WM were the same in control subjects and patients.

Discussion

In our study, we evaluated data from patients with PKU by using an MR technique that has been applied to other conditions affecting myelin, such as multiple sclerosis (13,25). A number of findings emerge from our study.

NAWM Exists in Two Forms

NAWM in patients with PKU exists in two forms: *(a)* that with normal characteristics on IW and T2-weighted MR im-

ages and long T2 MR maps and *(b)* that with increased signal intensity on long T2 MR maps. To our knowledge, this finding has not been previously reported. The IW-T2 NAWM appears to show more severe reductions in MWF than does the IW-T2–long T2 NAWM, and further studies are needed to explore the significance of this finding.

NAWM and Diffuse WM Lesions Both Show Changes in RWC

We found that IW-T2–long T2 NAWM, IW-T2 NAWM, and diffuse WM lesions showed increases in RWC compared with normal WM (Fig 2). Earlier publications (3,26) were focused mostly on changes occurring in areas of visible WM abnormalities, although one later article (5) included measurements from a region that included "predominantly normal white matter." We have focused on delineating NAWM from diffuse WM lesions. We found increases in RWC in IW-T2–long T2 NAWM that ranged from 1.9% to 3.9% relative to normal WM (mean ± standard error, 2.9% ± 0.4), which are slightly larger values than those previously reported for NAWM in other diseases, such as multiple sclerosis (13), where mean RWC was 2.2% ± 0.5 higher in NAWM of patients than WM in control subjects. Since some of the putative mechanisms of neurotoxicity of phenylalanine (27,28) involve global changes in neurotransmitter synthesis and oligodendrocyte function, it is not surprising that abnormalities are present even in WM that appears normal on conventional MR images. Changes in NAWM are more sensitive measures of disease activity in

conditions such as multiple sclerosis (14), and it is possible that focusing on the subtypes of IW-T2–long T2 and IW-T2 NAWM in PKU may improve the correlation between imaging techniques and cognitive outcomes. Thus, we have confirmed that NAWM in patients with PKU is not actually normal, and future studies on the relevance of these changes in RWC to clinical outcomes are needed.

Reduced MWF in Patients Relative to Control Subjects

There is a significant reduction in the MWF in all WM structures in patients with PKU relative to normal WM, regardless of their MR appearance (Fig 3). This reduction is particularly prominent in IW-T2 NAWM, which suggests that these brain regions may be an active site of disease activity, but further work is required to confirm this hypothesis. Results of a previous study (11) have demonstrated a good correlation between MWF measurements and luxol fast blue staining for myelin, and we believe that the observed reduction in MWF can be interpreted as a reduction in total myelin content. There are four potential physiologic hypotheses to explain this apparent reduction in myelin content:

The first is that the reduction in MWF reflects a reduction in myelin content secondary to axonal loss. We think this is unlikely because MR spectroscopy typically shows reduced levels of *N*-acetylaspartate in the presence of axonal loss (29). Consistent with results of other studies (3,5), we did not find reduced levels of *N*-acetylaspartate in pa-

tients with PKU. The minor increase we found in *N*-acetylaspartate has not been found by other authors (4,5) and was of borderline statistical significance ($P = .047$), making the clinical relevance of this finding difficult to interpret.

The second hypothesis is that because the MWF is a ratio of water with a T2 of less than 50 msec relative to total water content, the observed decrease in MWF may be caused by an increase in total water content alone. However, this seems unlikely, because a 4.1% increase in RWC (and a 12% increase in brain volume) would be required to account for the observed change in MWF (13).

The third hypothesis is that the reduction in MWF reflects a reduction in myelin content owing to decreased formation of myelin. This hypothesis would be consistent with rat models of PKU (30) that have shown a reduction in levels of brain enzymes required for myelinogenesis in hyperphenylalaninemic rats.

The fourth hypothesis is that the reduction in MWF reflects a reduction in myelin content secondary to demyelination. Although we did not see spectroscopic markers of active demyelination, such as increased levels of choline or a lipid peak (29), it is possible that the T2 technique may be more sensitive to this change than is MR spectroscopy. Pietz and colleagues (31), by using phosphorus 31 MR spectroscopy, have reported that cerebral energy metabolism worsens under the conditions of phenylalanine loading. It is possible that this altered energy metabolism may contribute to the formation of structurally altered and less stable myelin (ie, dysmyelination) as has previously been proposed (32,33).

Prolonged T2 Peak Exists in Diffuse WM Lesions in Patients

A water reservoir with a markedly prolonged T2 peak was identified in diffuse WM lesions in patients with PKU. Prolonged T2 times have also been identified in earlier articles (3,5). In our study, this reservoir accounted for approximately 9% of the total water content of the diffuse WM lesions. RWC in diffuse WM lesions, however, was only

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Table 3

Metabolite Concentrations at MR Spectroscopy in 13 Patients with PKU and 13 Control Subjects

| Metabolite | Mean Concentration (mmol/L) | | P Value |
	Patients	Control Subjects	
Choline	1.58 (0.05)	1.64 (0.08)	.59
Creatine	6.98 (0.12)	7.03 (0.23)	.85
N-acetylaspartate	10.0 (0.22)	9.31 (0.27)	.047
Myoinositol	3.59 (0.13)	4.16 (0.11)	.003

Note.—Data in parentheses are the standard error.

2.5% and 2.8% higher than that in normal WM in the major and minor forceps, respectively. Thus, the redistribution of water into this reservoir exceeds the magnitude of the increase in water content. The nature of the water in this reservoir is not known, but its T2 peak is separate from that of cerebrospinal fluid. Blood also has a prolonged T2 peak, but findings in the literature suggest that there is no difference in cerebral blood flow between patients with PKU and control subjects (34), so this reservoir cannot be attributed to blood.

Shift of Water into Extracellular Reservoir with Prolonged T2 Time May Explain Spectroscopy Findings

We have found a 14% reduction in myoinositol in patients with PKU relative to control subjects. A similar trend has been shown by other authors (8), although it has not always reached statistical significance (35) nor has it been found in all studies (5,26). We do not believe that the changes we observed in myoinositol are related to changes in T2 metabolites, because a 60% decrease in the T2 for myoinositol would be required to account for the observed change in concentration of myoinositol between patients and control subjects. Since myoinositol acts as an intracellular osmolyte (36), it is possible that the reduction of myoinositol results in a redistribution of water into this extracellular reservoir. Because our MR spectroscopy data were acquired from a single voxel that includes both lesions and NAWM, we cannot determine if the reduction in myoinositol concentration exists primarily in diffuse WM lesions and IW-T2 NAWM or whether it is a global reduction.

Dezortová et al (35) found that the apparent diffusion coefficient for water was smaller in PKU lesions than in NAWM. Kono et al (37) also found a restricted apparent diffusion coefficient in deep cerebral WM regions in patients with PKU that was inversely correlated with blood phenylalanine levels. However, this latter group included areas of both normal and high signal intensity on T2-weighted images when calculating the apparent diffusion coefficient val-

ues, so they cannot fully distinguish diffuse WM lesions from NAWM as we have done in our study (37). These results are consistent with our observation of a long T2 water reservoir (possibly extracellular in nature) in diffuse WM lesions and IW-T2 NAWM. We suggest that the smaller apparent diffusion coefficient in PKU lesions arises because intracellular water, which is the dominant reservoir of water in brain tissue, experiences a more restricted environment in lesions than in NAWM. We further suggest that, in lesions, there is a link between the decrease in the concentration of the intracellular osmolyte, myoinositol, the formation of an extracellular water reservoir with a prolonged T2 time, and water mobility changes in the intracellular water.

Our study had limitations. The location of the spectroscopy voxel differs from the site of T2 measurement, which limits our ability to directly relate the changes we found by using these two techniques. Also, our data are focused on relative rather than absolute water contents. Despite these limitations, we have used our MR imaging technique to show consistent changes in RWC and MWF in patients with PKU relative to control subjects. We have shown that WM in patients with PKU that appears normal at conventional MR imaging is not normal but demonstrates increases in RWC and reductions in MWF, and, in some areas, a water reservoir is present with a prolonged T2 relaxation time. This reservoir is present in diffuse WM lesions and may be related to the reduced concentration of the osmotically active metabolite myoinositol. Further investigations are needed regarding the relationship between these MR findings and the cognitive status of patients with PKU and how this multiecho T2 relaxation technique can best be used to identify patients at risk for progressive neurologic damage from this disease.

References

1. Koch R, Moats R, Guttler F, Guldberg P, Nelson M. Blood-brain phenylalanine relationships in persons with phenylketonuria. Pediatrics 2000;106:1093–1096.

2. Brenton DP, Pietz J. Adult care in phenylketonuria and hyperphenylalaninaemia: the relevance of neurological abnormalities. Eur J Pediatr 2000;159(suppl 2):S114–S120.

3. Bick U, Ullrich K, Stöber U, et al. White matter abnormalities in patients with treated hyperphenylalaninaemia: magnetic resonance relaxometry and proton spectroscopy findings. Eur J Pediatr 1993;152:1012–1020.

4. Novotny EJ, Avison MJ, Herschkowitz N, et al. In vivo measurement of phenylalanine in human brain by proton nuclear magnetic resonance spectroscopy. Pediatr Res 1995;37:244–249.

5. Pietz J, Kreis R, Schmidt H, Meyding-Lamadé UK, Rupp A, Boesch C. Phenylketonuria: findings at MR imaging and localized in vivo H-1 MR spectroscopy of the brain in patients with early treatment. Radiology 1996;201:413–420.

6. Pietz J, Kreis R, Rupp A, et al. Large neutral amino acids block phenylalanine transport into brain tissue in patients with phenylketonuria. J Clin Invest 1999;103:1169–1178.

7. Kreis R. Comments on in vivo proton magnetic resonance spectroscopy in phenylketonuria. Eur J Pediatr 2000;159(suppl 2): S126–S128.

8. Johannik K, Van Hecke P, Francois B, et al. Localized brain proton NMR spectroscopy in young adult phenylketonuria patients. Magn Reson Med 1994;31:53–57.

9. Weglage J, Wiedermann D, Denecke J, et al. Individual blood-brain barrier phenylalanine transport determines clinical outcome in phenylketonuria. Ann Neurol 2001;50:463–467.

10. Whittall KP, MacKay AL, Graeb DA, Nugent RA, Li DK, Paty DW. In vivo measurement of T2 distributions and water contents in normal human brain. Magn Reson Med 1997;37:34–43.

11. Moore GR, Leung E, MacKay AL, et al. A pathology-MRI study of the short-T2 component in formalin-fixed multiple sclerosis brain. Neurology 2000;55:1506–1510.

12. Laule C, Leung E, Li DK, et al. Myelin water imaging in multiple sclerosis: quantitative correlations with histopathology [abstr]. In: Proceedings of the 12th Meeting of the International Society for Magnetic Resonance in Medicine. Berkeley, Calif: International Society for Magnetic Resonance in Medicine, 2004; 461.

13. Laule C, Vavasour IM, Moore GR, et al. Water content and myelin water fraction in multiple sclerosis: a T2 relaxation study. J Neurol 2004;251:284–293.

14. Sarchielli P, Presciutti O, Pelliccioli GP, et al. Absolute quantification of brain metabolites by proton magnetic resonance spectroscopy in normal-appearing white matter of multiple sclerosis patients. Brain 1999;122:513–521.

15. Laule C, Whittall KP, MacKay AL. Shortening the acquisition time of a 48 echo T2 relaxation pulse sequence by varying TR across k-space [abstr]. In: Proceedings of the Ninth Meeting of the International Society for Magnetic Resonance in Medicine. Berkeley, Calif: International Society for Magnetic Resonance in Medicine, 2001; 896.

16. Whittall KP, MacKay AL. Quantitative interpretation of NMR relaxation data. J Magn Reson 1989;84:64–71.

17. Fatouros PP, Marmarou A. Use of magnetic resonance imaging for in vivo measurements of water content in human brain: method and normal values. J Neurosurg 1999;90:109–115.

18. Griffin CM, Chard DT, Parker GJ, Barker GJ, Thompson AJ, Miller DH. The relationship between lesion and normal appearing brain tissue abnormalities in early relapsing remitting multiple sclerosis. J Neurol 2002;249:193–199.

19. Brief EE, Whittall KP, Li DK, MacKay AL. Proton T2 relaxation of cerebral metabolites of normal human brain over large TE range. NMR Biomed 2005;18:14–18.

20. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med 1993;30:672–679.

21. Brief EE, Whittall KP, Li DK, MacKay AL. Proton T1 relaxation times of cerebral metabolites differ within and between regions of normal human brain. NMR Biomed 2003;16:503–509.

22. Brief E, Whittall K, Li DK, MacKay AL. Absolute quantification in MRS with an external standard: there is no invisible water [abstr]. MAGMA 2000;11(suppl 1):188.

23. Moll R. Metabolite concentrations and myelin water fraction: correlations and trends within white matter measured with magnetic resonance spectroscopy and T2 relaxation techniques [master's thesis]. Vancouver, Canada: University of British Columbia, 2002; 41–44.

24. Zhao GJ, Li DK, Cheng Y, Wang XY, Paty DW; and the UBC MS/MRI research group. Active multiple sclerosis lesions occur in dirty-appearing white matter as detected by serial magnetic resonance imaging [abstr]. Ann Neurol 1998;44:465.

25. Whittall KP, MacKay AL, Li DK, Vavasour IM, Jones CK, Paty DW. Normal-appearing white matter in multiple sclerosis has heterogeneous, diffusely prolonged T2. Magn Reson Med 2002;47:403–408.

26. Pietz J, Kreis R, Boesch C, Penzien J, Rating D, Herschkowitz N. The dynamics of brain concentrations of phenylalanine and its clinical significance in patients with phenylketonuria determined by in vivo 1H magnetic resonance spectroscopy. Pediatr Res 1995;38:657–663.

27. Donlon J, Levy H, Scriver CR. Hyperphenylalaninemia: phenylalanine hydroxylase deficiency. In: Scriver CR, Beaudet AC, Valle D, et al, eds. The metabolic and molecular basis of inherited disease, online edition. New York, NY: McGraw-Hill, 2001.

28. Martin JJ, Schlote W. Central nervous system lesions in disorders of amino-acid metabolism. J Neurol Sci 1972;15:49–76.

29. Degaonkar MN, Khubchandhani M, Dhawan JK, Jayasundar R, Jagannathan NR. Sequential proton MRS study of brain metabolite changes monitored during a complete patho-logical cycle of demyelination and remyelination in a lysophosphatidyl choline-induced experimental demyelinating lesion model. NMR Biomed 2002;15:293–300.

30. Burri R, Steffen C, Stieger S, Brodbeck U, Colombo JP, Herschkowitz N. Reduced myelinogenesis and recovery in hyperphenylalaninemic rats. Mol Chem Neuropathol 1990;13:57–69.

31. Pietz J, Rupp A, Ebinger F, et al. Cerebral energy metabolism in phenylketonuria: findings by quantitative in vivo 31P MR spectroscopy. Pediatr Res 2003;53:654–662.

32. Hommes FA, Matsuo K. On a possible mechanism of abnormal brain development in experimental hyperphenylalaninemia. Neurochem Int 1987;11:1–10.

33. Vermathen P, Pietz J, Boesch C, Kreis R. Mapping human cerebral water compartments based on simultaneously acquired T1 and T2 data: evidence of dysmyelination in phenylketonuria [abstr]. In: Proceedings of the 12th Meeting of the International Society for Magnetic Resonance in Medicine. Berkeley, Calif: International Society for Magnetic Resonance in Medicine, 2004; 11.

34. Hasselbalch S, Knudsen GM, Toft PB, et al. Cerebral glucose metabolism is decreased in white matter changes in patients with phenylketonuria. Pediatr Res 1996;40:21–24.

35. Dezortová M, Hájek M, Tintěra J, Hejcmanová L, Syková E. MR in phenylketonuria-related brain lesions. Acta Radiol 2001;42:459–466.

36. Haussinger D, Laubenberger J, vom Dahl S, et al. Proton magnetic resonance spectroscopy studies on human brain myo-inositol in hypo-osmolarity and hepatic encephalopathy. Gastroenterology 1994;107:1475–1480.

37. Kono K, Okano Y, Nakayama K, et al. Diffusion-weighted MR imaging in patients with phenylketonuria: relationship between serum phenylalanine levels and ADC values in cerebral white matter. Radiology 2005;236:630–636.