Brain tissue concentrations of phenylalanine (Phe) can be measured by proton magnetic resonance spectroscopy in patients with phenylketonuria (PKU). In combination with oral Phe challenges, the kinetics of Phe transport at the blood–brain barrier can be characterized. Weglage and colleagues reported interindividual differences and an association of such kinetic parameters with IQ and magnetic resonance imaging (MRI) visible white matter changes in a group of 15 PKU patients. It was interpreted to indicate that “interindividually different blood–brain barrier transport characteristics [...] are major causative factors for clinical outcome in PKU.”

The results of Weglage and colleagues contradict a recent study by Rupp and colleagues, which yielded a close linear correlation was found by the researchers. In their recent work, the corresponding preload ratio was 3.8 ± 1.1. This suggests a systematic difference between the present and earlier studies (Fig). The data of the two patients with lowest $K_{app}$ and highest $T_{max}/V_{met}$ (no.1 and 2), and hence largest influence on the apparent “individuality” of the kinetic parameters, already were included in earlier studies (nos. 8 and 9 in Möller and colleagues). These two patients, who previously have been characterized as “typical” PKU patients, now seem to represent “particular” patients compared with the remaining 13 patients (eg, $T_{max}/V_{met}$ is >6 standard deviations above the mean of the 13 new patients), whereas the kinetic parameters of three previously “atypical” patients fit well with the new data set. Thus, the statistics in the recent article appear to be based on an inhomogeneous sample (Fig) composed of 13 recently investigated patients and 2 patients added from an earlier study. If the latter are excluded from analysis, the correlations of IQ with $K_{app}$ ($r = 0.23, p = 0.46$) and with $T_{max}/V_{met}$ ($r = -0.31, p = 0.31$) decrease, corroborating the results of Rupp and colleagues.

In summary, clinically significant interindividual differences in blood–brain barrier Phe transport and an influence of kinetic characteristics determined in adult life on outcome parameters is not confirmed for most “typical” PKU patients. The conclusion that the reported observations ultimately could lead to individual dietary recommendations is currently not tenable.
to-noise ratio of the Phe signal under such conditions, esti-
sists variability, even when comparing studies from the
brain Phe at concentrations well below 1mmol/L cause con-
centrations estimates from different groups as examined
centrations will not persist beyond some threshold of
concentration significant saturation occurs in individual patients,
physiological understanding. Hence, we do not see sufficient
reason to exclude patients from analysis. Preselection of pa-
tients to achieve a homogeneous sample may be appropriate
for a metabolic characterization of most PKU patients. How-
ever, it is not likely to lead to an understanding of the heter-
egeneity in the clinical outcome, which is known to vary sig-
ficantly in a subgroup of patients. Nevertheless, the numbers of patients included in both our most recent study5
and the one by Rupp and colleagues12 are low, and additional research certainly is indicated to clarify potential inconsist-
encies among the results.

There is no doubt that all large neutral amino acids are
transported across the blood–brain barrier by a single facili-
tated system, which is stereospecific and follows Michaelis-
Menten kinetics. Numerous studies in animals and cell cul-
tures have underlined that different affinities do exist for the
large neutral amino acids to the same carrier system. Results
of previous investigations by Möller and colleagues4,4 sug-
gested a saturation kinetics for Phe at this carrier system.
This is consistent with kinetic data measured in hyperphe-
nylalaninemic rabbits.8 In addition, direct support for this
hypothesis arises from permeability surface area products for
amino acid transport into human brain measured by the
double-indicator method.9 Furthermore, Pietz and col-
leagues10 demonstrated that Phe influx into the brain via the
L-type amino acid carrier can be competitively blocked by
supplementing high doses of all other large neutral amino
acids. Although it is an open question at which Phe concen-
tration significant saturation occurs in individual patients,
there is no doubt from basic physiological understanding
that a close linear regression for blood and brain Phe con-
centrations will not persist beyond some threshold of [Phe]blood. Again, more investigations are clearly warranted
to answer this question.

Intracerebral Phe levels measured by magnetic resonance
spectroscopy under steady state conditions in PKU patients
clearly remained below blood concentrations with ratios
[Phe]blood to [Phe]brain varying between 3.45 and 4.47 in the
study of Rupp and colleagues7 and between 1.7 and 5.6 in
our recent study.5 Our measurements compare very well
with a recent study in 29 PKU patients by Koch and col-
leagues,11 indicating that interindividual differences in
[Phe]brain in patients with similar blood concentrations of
Phe are not limited to a few exceptional cases but may be
more common. However, some inconsistencies between the
results, reported by Rupp and colleagues,7 Weglage and col-
leagues,5 and Koch and colleagues11 remain currently unex-
plained. Further research is absolutely needed.

There is no doubt that the quality of dietary control dur-
ing the first years of life is the most important factor for the

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DOI: 10.1002/ana.10289

Reply
Josef Weglage, MD, PhD,1 Dirk Wiedermann, PhD,2
Reinhold Feldmann, PhD,1 Kurt Ullrich, MD,3
and Harald E. Möller, PhD4

Different clinical outcome despite comparable dietary control
is well known in patients with phenylketonuria (PKU).1 Single
case reports have described untreated patients with classic
PKU and blood phenylalanine (Phe) levels consistently above
1.2mmol/L and with normal intelligence.2 Exact mechanisms
for this most interesting phenomenon are still not well
understood.

Recently, researchers at the University of Bern/Heidelberg,
Yale, and University of Münster (cited according to Möller
and colleagues5) demonstrated independently that in vivo
proton magnetic resonance spectroscopy offers a novel strategy
for quantifying intracerebral Phe concentrations. Slightly
different examination techniques (eg, point resolved spectro-
scopy versus stimulated echo acquisition mode) and, more
importantly, different strategies used for data evaluation
probably contribute to some systematic inconsistencies in the
concentrations estimates from different groups as examined
recently by Krei.5 Regarding potential interstudy inconsist-
encies, we have repeatedly pointed out that current limita-
tions of the experimental technique used for quantifying
brain Phe at concentrations well below 1mmol/L cause consider-
able variability, even when comparing studies from the
same laboratory.2,4,5 Because of the notoriously poor signal-
to-noise ratio of the Phe signal under such conditions, esti-
mates of [Phe]brain derived from nuclear magnetic resonance
spectroscopy are associated with relatively large errors. These
uncertainties in the [Phe]brain data unavoidably lead to sub-
stantial standard deviations of the estimated kinetic parame-
ters by error propagation.2–4

The figure presented by Pietz and colleagues6 might be
misleading, suggesting that our kinetic analysis5 was based on
single spectroscopic measurements under steady state condi-
tions. Thus, note that all kinetic parameters were, in fact,
extracted from oral loading experiments and linear fits of the
observed time-dependent variations of blood and brain Phe
concentrations. Consequently, potential interstudy variability
of [Phe]brain is not an issue for the individual time courses,
which were used in calculations of the parameters \(K_{\text{app}}\) and
\(T_{\text{max}}/T_{\text{app}}\) and our subsequent analysis.

Because the patient group examined in our last study5 is
relatively small, nonparametric tests (rank correlations ac-

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patients’ development. In addition, some studies suggest that the severity of magnetic resonance imaging changes is related to blood Phe levels during weeks to months before investigation. However, correlations between blood Phe levels and different parameters of clinical outcome generally were found to be low.\(^1\) This might be explained by our observation that patients’ brain Phe levels may be different despite comparable blood Phe levels.

Finally, in view of the discussion of potential error sources including such issues as the small patient population, we feel the results were interpreted with sufficient caution: ‘… we hypothesize that, in addition to the quality of diet during the first decade of life, favorable transport parameters may additionally protect the brain from uptake of high amounts of the neurotoxin Phe. … BBB Phe transport and \([\text{Phe}]_{\text{brain}}\) seem to be important factors for individual clinical outcome in PKU. … Our observations may ultimately lead to individual dietary recommendations in the future; however, numerous questions remain to be answered …’.\(^5\)

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DOI: 10.1002/ana.10289

Mild Cognitive Impairment and the Cholinergic Hypothesis: A Very Different Take on Recent Data

Martin Sarter, PhD, and John P. Bruno, PhD

In his recent editorial, Morris\(^1\) concluded that the very interesting data presented by DeKosky and colleagues\(^2\) challenge the hypothesis that decline in the integrity of basal forebrain cholinergic projections is associated with the emergence of cognitive impairments associated with the initial stages of Alzheimer’s disease (AD). We take issue with this interpretation and, in the context of other recent data, suggest that, in fact, quite the opposite is true: the case for an early involvement of the cholinergic system in the age-related cognitive decline has never been stronger.

The main finding by DeKosky and colleagues\(^2\) indicates that cortical choline acetyltransferase (ChAT) activity is unchanged in subjects with mild cognitive impairment (MCI) when compared with subjects with no cognitive impairment (NCI) and, in fact, is increased in hippocampal regions and the superior frontal cortex. As DeKosky and colleagues\(^2\) point out, ChAT activity does not represent the rate-limiting step in the synthesis and release of acetylcholine (ACh). Changes in ChAT activity likely indicate substantial changes in the density of presynaptic cholinergic neurons. Although DeKosky and colleagues\(^2\) and Morris appear to consider the absence of decreases in ChAT activity in MCI an unexpected result, this result is predicted by previous studies indicating no decline in the number of ChAT-immunoreactive neurons in the basal forebrain of subjects with MCI.\(^3\)

There is now ample evidence for the hypothesis that cholinergic neurons in MCI are not normally regulated. Mufson and colleagues\(^4,5\) reported that in MCI the number of neurons in the nucleus basalis showing immunoreactivity for trkA, the high-affinity receptor for nerve growth factor (NGF), as well as neurons immunoreactive for the low-affinity p75 NGF receptor, is significantly decreased when compared with NCI. Moreover, the numbers of immunoreactive neurons in MCI were statistically similar to the low number of trkA- or p75-immunoreactive neurons counted in AD.\(^4,5\) In situ hybridization further confirmed that the number of neurons expressing trkA is decreased in MCI and is indistinguishable from Alzheimer’s disease.\(^6\) Furthermore, the number of basalis neurons bearing NGF receptors correlated with the cognitive status of the subjects in these reports.\(^4,6\) Finally, in animal studies, ChAT activity changes are, as expected, a poor predictor of changes in the regulation of ACh release, specifically for the capacity of (residual) cholinergic neurons to respond to behavioral or pharmacological challenges.\(^7\)

The exact status of cholinergic transmission in the forebrain of subjects with MCI is unclear. Although it is unlikely that any ex vivo histochemical or neurochemical method might be capable of showing such changes, emerging positron emission tomography\(^8\) or single-photon emission
computed tomography methods to monitor cholinergic activity may provide insights into the functional status of cholinergic neurons in MCI. The extensive experimental evidence provides overwhelming support for the hypothesis that disruption of trophic factor support, as suggested by Morris, but instead provide increasingly impressive support for the hypothesis that possibly early abnormalities in the regulation of the cholinergic system trigger cognitive limitations that per se, and even more so in conjunction with an accelerating decline in cholinergic functioning, contribute to the emergence of dementia.11–15

Finally, the therapeutic limitations of cholinesterase inhibitors may reflect the limited efficacy of these drugs to enhance or reinstate phasic cholinergic transmission and/or the possibility that postsynaptic signaling mechanisms are also disrupted, rather than rejecting hypotheses about the role of abnormal cholinergic transmission in the manifestation of age-related cognitive impairments, as suggested by Morris. Clearly, “the cholinergic hypothesis” remains a simplistic and likely incomplete account of the neuronal bases of cognitive decline, particularly if the discussion remains focused on cell loss and ignores changes in cholinergic signal regulation. However, the available evidence from subjects with MCI and Alzheimer’s disease, together with the extensive experimental literature, indicates that it remains one of the most viable hypotheses in this field.

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DOI: 10.1002/ana.10308