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Phenylalanine levels of 6–10 mg/dl may not be as benign as once thought

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Results of a longitudinal study of children treated early and continuously for phenylketonuria (PKU) indicated that those children whose plasma phenylalanine (Phe) levels were approximately 3–5 times normal (6–10 mg/dl; *levels previously considered safe in the US*) were impaired in cognitive functions dependent on prefrontal cortex. In particular, the children had difficulty when required to hold information in the mind and, at the same time, exercise inhibitory control to resist doing what might be their first inclination. The deficits were evident in relation to each of several comparison groups and at all three age ranges (infants, toddlers and young children). The deficits appeared to be selective in that the same children who were impaired on the prefrontal cortex tests performed normally on the control tests. Since most of the control tasks tap functions dependent on parietal cortex or the medial temporal lobe, these results suggest that those functions are spared. To investigate the biological mechanism causing these cognitive deficits, we created an animal model of early-treated PKU. The results indicated that rats whose plasma Phe levels were mildly, but chronically, elevated had cognitive deficits (impaired performance on a behavioral task dependent on frontal cortex (delayed alternation)) and neurochemical changes (most notably, reduced dopamine metabolism in frontal cortex). □

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Phenylketonuria (PKU) is an inborn error in the metabolism of phenylalanine (Phe) to tyrosine due to the absence or markedly reduced activity of phenylalanine hydroxylase in the liver. Its incidence in the US is roughly 1 in every 10 000 children (1). It is most commonly caused by mutations of the gene in chromosome 12 (12q22–12q24.1) that codes for phenylalanine hydroxylase (2–4).

The inborn error in the metabolism of Phe in children with PKU results in a build-up of Phe in the blood stream (5) and often lower blood levels of tyrosine. Treatment consists of lowering dietary Phe intake. Dietary treatment reduces the build-up of Phe but does not eradicate it because of the need to balance reducing intake of Phe with the body's need for protein. The mild imbalance in the Phe : tyrosine ratio found in PKU children on a low-Phe diet results in mildly reduced levels of tyrosine reaching the brain for the following reasons: (1) Phe and tyrosine compete for the same limited supply of transporter proteins to cross the blood–brain barrier (6–10); and (2) these protein carriers have a higher affinity for Phe than tyrosine (9, 10). Because the ratio of Phe to tyrosine is only *moderately* increased in those on dietary treatment for PKU, the decrease in the amount of tyrosine reaching the brain is correspondingly modest.

Dopamine metabolism in prefrontal cortex is more sensitive to a mild reduction in tyrosine than dopamine metabolism in most other areas of the brain. Most areas of the brain receiving dopaminergic input are unaffected by modest changes in the level of tyrosine. Prefrontal cortex is an exception because the dopaminergic neurons innervating it differ from most other dopaminergic neurons in the brain. They fire faster, turn over dopamine faster and tyrosine hydroxylase isolated from prefrontal cortex exists primarily in its activated state (11–15). This makes prefrontal cortex acutely sensitive to even a small change in tyrosine levels (13, 14, 16). Reductions in tyrosine too small to affect dopaminergic systems elsewhere in the brain have been shown to profoundly affect prefrontal cortex (17). Moreover the cognitive functions dependent upon prefrontal cortex are acutely sensitive to any reduction in dopamine in prefrontal cortex (18–19).

For these reasons it seemed plausible that the mild imbalance in the Phe : tyrosine ratio in the plasma of children treated for PKU might well result in deficits in the cognitive abilities dependent on prefrontal cortex without other cognitive abilities or other areas of the brain being affected.

Longitudinal study of children treated early and continuously for PKU

Here are highlighted a few of the results for the 37 children with PKU who have been followed for 4 years (20). These children were maintained on a low-

Phe diet from ⩽14 days of age. The other children who were followed over this time were 25 children with mild hyperphenylalaninemia, 30 subjects matched to the PKU and hyperphenylalaninemic children on a variety of background and health variables (matched controls) and 21 siblings of the PKU and hyperphenylalaninemic children (including a pair of twins discordant for PKU). Subjects aged 6–12 months were tested on four tasks at 1-month intervals. Subjects aged 15–30 months were tested on five tasks at 3-month intervals. Subjects aged 3.5–7 years were tested on 10 tasks at 6-month intervals. In addition, 512 children from the general population were tested (25 longitudinally, 87 cross-sectionally). Subjects were tested on multiple tasks linked to frontal cortex (to obtain converging evidence) and on multiple control tasks that do not require frontal cortex (to assess specificity). Most of the control tasks required the functions of parietal cortex or the medial temporal lobe. Behavioral testers were kept blind to the children's Phe levels. IQ was assessed at 4 years. All subjects tested had IQs between 80 and 125. Plasma Phe levels in the PKU subjects were generally checked every 2–4 weeks from 6 to 12 months and every 1–3 months thereafter. Examples of the tasks on which PKU children with plasma Phe levels 3–5 times normal were impaired are:

As infants, one of the tasks on which subjects treated early and continuously for PKU were impaired was AB̄, a test linked to prefrontal cortex function in both adult and infant monkeys (21, 22). On the AB̄ task (23), the subject watches as a reward is hidden in one of two hiding places that differ in left–right location only, a brief delay is imposed during which visual fixation of the hiding places is prevented and then the subject is allowed to reach. Errors typically occur on reversal trials (trials where the subject was correct on the previous trial and the reward is hidden in the other hiding location on the current trial). Infants with PKU whose mean plasma Phe levels were 6–10 mg/dl, or whose mean Phe levels were lower but the levels showed marked fluctuation, could withstand significantly briefer delays on the AB̄ task than other infants of the same age (whether matched controls, sublings or children from the general population).

As toddlers, the children treated early and continuously for PKU whose mean plasma Phe levels were 6–10 mg/dl were impaired on AB̄ with invisible displacement. Here the subject watches the reward being hidden in a container at the midline, and then watches as that container is moved to the right or the left; a screen is lowered for a 5-s delay; then the screen is raised revealing two identical containers that differ only in left–right location. As on the infant AB̄ task, side of hiding is reversed after the subject is correct twice in a row; errors typically occur on the reversal trials. Toddlers whose mean Phe levels were 6–10 mg/dl made significantly more errors on these reversal trials than other children of the same age (whether matched controls, sublings, other PKU children with lower Phe levels or children from the general population); the delay was held constant.

As young children, one of the tasks on which subjects treated early and continuously for PKU whose mean plasma Phe levels were 6–10 mg/dl performed significantly worse was a day–night Stroop-like task than matched controls, siblings, other PKU children with lower Phe levels and children from the general population. On the day–night task, subjects must say "day" when shown a black card containing a picture of the moon and stars, and say "night" to a white card containing a bright sun (24). Thus subjects must remember two rules and must inhibit saying what the cards actually represent. Children whose Phe levels were 6–10 mg/dl were correct on significantly fewer of the 16 trials. They had no difficulty, however, on the control condition that removes the inhibitory requirement. Here subjects say "day" to one abstract design and "night" to another; PKU children, regardless of their Phe level, performed as well as all other children.

The children treated for PKU were not impaired at any age on any of the control tasks, regardless of their plasma Phe levels. These control tasks included a spatial discrimination task, recognition memory tasks, visual paired comparison, delayed non-matching to sample, global–local spatial processing and line bisection. Their normal performance here suggests that the functions of the parietal cortex and then medial temporal lobe appear to be spared.

An animal model of early-treated PKU

A technique developed by Greengard et al. to model untreated PKU (25–27), was modified to model the milder, treated condition. Rat pups were injected daily with α-methylphenylalanine, an inhibitor of phenylalanine hydroxylase (the enzyme that metabolizes Phe), as well as a small supplement of Phe (28). The pups were impaired on delayed alternation, a behavioral task sensitive to the functions of the prefrontal cortex. No effect on norepinephrine was found anywhere in the brain, and dopamine metabolism was markedly reduced in frontal cortex, and there was an effect on the serotonergic system as well. The only neurochemical effect significantly correlated with every measure of delayed alternation performance was reduced dopamine metabolism in prefrontal cortex. The areas studied outside of frontal cortex were the caudate-putamen and nucleus accumbens, the areas of the brain richest in dopamine.

Visual contrast sensitivity in children treated early and continuously for PKU

If we are correct about why prefrontal dopamine metabolism is disproportionately affected by a moderate

increase in plasma levels of Phe (moderate increases in the Phe : tyrosine ratio in blood result in modest decreases in the amount of tyrosine reaching the brain and modest decreases in tyrosine levels in the brain disproportionately affect those dopamine neurons that fire most quickly and turnover dopamine most rapidly) then other dopamine neurons that share these characteristics should also be affected.

Retinal dopamine neurons share these characteristics. They fire at a rapid rate and have an extremely high rate of dopamine turnover. Also, tyrosine hydroxylase in the retina is in a highly activated state (29–31). Reduced dopamine in the retina is associated with impaired contrast sensitivity. Patients with Parkinson's disease, who have reduced levels of dopamine, have impaired sensitivity to contrast (32). We predicted, therefore, that children treated early and continuously for PKU would be found to have impaired contrast sensitivity, even though their plasma Phe levels remained under what has been considered excellent control. Our results indicate that this is, in fact, the case (33).

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