Phenylketonuria: questioning the gospel

WB Hanley

Phenylketonuria (PKU) was first described over 70 years ago, treatment was developed 50 years ago and universal newborn PKU screening was introduced 40 years ago. Phenylalanine-restricted dietary treatment has prevented mental retardation in thousands of individuals worldwide. We acknowledge, however, that there is still much to learn in the field. The incidence of mental retardation in untreated PKU is likely to be considerably less than the original estimates. Since dietary control is suboptimal in late childhood, adolescence and adulthood, alternative methods of treatment are being explored. These include large neutral amino acids, phenylalanine ammonia lyase, tetrahydrobiopterin and gene replacement. Evidence has surfaced that the semisynthetic, low-protein diet used to treat PKU may be deficient in certain important nutrients. Maternal PKU treatment may be successful even if initiated as late as 8–10 weeks into pregnancy. A plea is made for the immediate establishment of adult treatment centers for PKU (and other inherited metabolic diseases) for long-term treatment, follow-up and research.

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The Hospital for Sick Children and the Faculty of Medicine, University of Toronto, Division of Clinical & Biochemical Genetics, Department of Paediatrics, 555 University Ave, Toronto, ON M5G 1X8, Canada
Tel.: +1 416 425 7846
Fax: +1 416 781 8515
whanley@sickkids.ca

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> *'Instead of marching on with perfect vision, science stumbles along behind leaders who occasionally take the wrong alley, after which they turn to other leaders who seem to know the way, then corrects itself again, until sufficient progress is made for the next generation to either thrust aside or build upon. In hindsight the path may look straight, running from ignorance to profound insight, but only because our memory for dead ends is so much worse than that of a rat in a maze'* [1].

Sir Peter Medawar once stated 'the intensity of the conviction that a hypothesis is true has no bearing on whether it is true or not' [2]. It has been claimed that the half-life of medical knowledge is 5 years – that is, 50% of what is looked on as 'the gospel' today will be proven wrong in 5 years. This anonymous statement is supported by Shekelle *et al.*, who reviewed 17 'Clinical Practice Guidelines' published by the US Agency for Health Care Research and Quality, still in circulation [3]: seven needed major updates, six needed minor updates, three were still valid and in one there was no conclusion. A half were outdated in 5.8 years.

Phenylketonuria (PKU), first described in 1934 by Folling [4], is a recessively inherited genetic metabolic disease where deficient function of the enzyme phenylalanine hydroxylase (PAH), which converts phenylalanine (phe) to tyrosine, results in high levels of phe in the body tissues – notably the blood and brain – and, if not detected in the neonate and treated immediately, can cause profound and permanent mental retardation. It is not yet clear how the elevated phe damages the brain. Treatment of PKU with a restricted-phe diet was first described in 1954 by Bickle *et al.* [5]. Universal newborn screening, introduced by Guthrie *et al.* in 1963 [6], has become widespread. The incidence of PKU varies from highs of one per 1700 births in eastern Turkey and one per 3500 in Ireland to lows of one per 150,000 in Finland and one per 100,000 in Japan; averaging one per 10,000 in Europe and Australia and one per 15,000 in North America [7]. We are constantly and vehemently reminded by

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the bioethicists and statisticians that no epidemiologically viable clinical trials were ever carried out for diet therapy [8] or for the newborn PKU screening initiative [9]. Since newborn screening evolved, various clinical phenotypes of PAH-deficiency PKU have emerged and are listed in TABLE 1. The third phenotype, non-PKU mild hyperphenylalaninemia (MHP) does not appear to damage the affected patients [10] or their offspring (even though blood phe levels are up to seven-times the upper limit of normal) [11]. Over 500 genotypes have been described. Genotype–phenotype correlation is inconsistent, perhaps affected by modifier genes. In most cases, however, one mild allele results in mild disease. An uncommon variant of persistent hyperphenylalaninemia (HPA), biopterin deficiency (or 'malignant') PKU [12], makes up 1–2% of the total, and involves malfunction of one of several biopterin-metabolizing enzymes (biopterin is a cofactor for the PAH complex). This variant needs additional treatment with tetrahydrobiopterin (BH4), folinic acid, levodopa/carbidopa and 5-hydroxytryptophan. When nubile women with PKU become pregnant, their fetuses are profoundly damaged if dietary treatment is not administered throughout pregnancy. These offspring suffer from the maternal PKU (MPKU) syndrome: microcephaly, mental retardation, intrauterine growth retardation, facial dysmorphism and congenital heart disease [13]. Of note, this constellation of signs and symptoms is virtually indistinguishable from fetal alcohol syndrome (FAS).

Treatment of PKU: restricted-phe dietary therapy

For the last 50 years, treatment of PKU has consisted of gradually more refined, phe-restricted diet therapy. Since phe is an essential amino acid, this diet must contain just enough phe to sustain protein anabolism but not an amount that results in toxic elevation of blood (and brain) levels. It usually consists of a synthetic, phe-free, amino acid formula or medical food with added essential nutrients plus a vegan–vegetarian diet of natural foods (no meat, eggs, bread, milk or cheese), which supplies just enough phe for normal growth and development. Monitoring of blood phe levels and nutritional parameters is carried out regularly. The recommended standard of practice is the 'phe-restricted diet for life' [14]. However, problems arise in older children, adolescents and adults, where deviations from the recommended diet results in loss of biochemical control. When diet control becomes suboptimal, these individuals do not suffer acute (biochemical/neurological) decompensation, such as that which occurs in many other metabolic diseases. This has led to questions such as do all adolescents and adults with PKU need continual, highly restricted, diet therapy? Are there other, more easily administered treatments?

Table 1. A classification of the phenotypes of PAH-deficiency PKU: based on plasma phenylalanine levels on an unrestricted diet.

Classical PKU	phe > 1200 µmol/l
Atypical/mild PKU	phe 600–1200 µmol/l
Non-PKU mild hyperphenylalaninemia	phe 150–599 µmol/l

PAH: Phenylalanine hydroxylase; Phe: Phenylalanine; PKU: Phenylketonuria.

The gospel

Over the years, a number of axioms have evolved concerning PKU and its treatment. We will call these 'the gospel' and discuss some of the ramifications as seen from our perspective. Here are some of the statements that are considered gospel by many:

- Virtually all untreated subjects with PKU become profoundly retarded
- The standard and only mode of treatment is diet for life
- Current available dietary formulations for PKU provide all the necessary nutrients
- Treatment of MPKU must always begin prior to conception to prevent MPKU embryopathy

We will explore each of these statements in turn and attempt to relate them to current and future treatment of PKU.

Incidence of mental retardation in undiagnosed PKU & its variants

In the 1950s, before treatment for PKU had been developed, most individuals with mental retardation were confined to large institutions. This made it relatively easy for interested scientists to determine how many had PKU; the figure was 1–2%. They could then roughly determine what the incidence would be in the general population. Their estimation was one in 25,000 [15]. When early universal screening data showed the incidence was one in 10,000 to one in 15,000, the scientists had no explanation except the possibility of underestimates from their earlier studies. It is quoted that 'only 1–2% of patients with untreated classical PKU have normal intellectual function' [15]. Citations to support this include Knox, who, in 1960, reviewed the world literature for published reports of untreated PKU, finding 466 subjects [16]. Of these subjects, 87% had an IQ below 40 and only 0.6% had IQs greater than 81. Similar surveys and results were reported by Paine in the USA [17], Partington in Canada [18] and Pitt in Australia [19]. Knox, however, recognized the bias of ascertainment (institutionalized patients) and noted that 'the possible existence of a substantial number of high-grade cases in the normal population has not been disproved' [16].

Many modern textbooks still quote the claims established by these original authors: McKusick, for example, states that 'normal mentality is very rare among patients with PKU who have not received dietary treatment' [20]. Levy *et al.*, however, took Knox's concerns to heart and screened blood samples from 250,000 normal adults in Massachusetts [21]. They found only three adults with PKU, all were mentally retarded, and concluded that 'among those with PKU who have not received dietary therapy, very few are mentally normal'. However, a later epidemiological review of this paper calculated that the statistical power was only 12% [22]. Machill *et al.* prospectively screened 233,663 pregnant women for PKU between 1972 and 1989 and

found 17 women with previously undiagnosed PKU [23]. They concluded that 20% of untreated classical PKU subjects have normal IQs. Berman *et al.* [24] and Koch *et al.* [25] tested all of the older, unscreened siblings of neonates diagnosed in the early days of newborn screening and each found 15 with (untreated) PKU; four (27%) of Berman's and three (20%) of Koch's patients had normal IQs. Levy *et al.*, between 1971 and 1981, tested 453,118 umbilical cord blood samples and found 22 previously undiagnosed, untreated women with PKU [26,27]. Of these, two had classical PKU, 11 had mild/atypical PKU and nine had MHP. The two with classical PKU had IQs of 45 and 94, the six women with mild/atypical PKU had a mean IQ of 97.3 (range [R]: 78–107; SD: 9.8) and the six with MHP had a mean IQ of 105.7 (R: 91–122; SD: 11.8). We found, in an earlier review of published reports, 24 women with untreated PKU diagnosed only after producing 47 offspring – 45 with profound MPKU embryopathy. The majority of these women had normal to near-normal IQs [28].

What is behind this phenotypic heterogeneity? Proton nuclear magnetic resonance spectroscopy (MRS) may give some answers. Weglage *et al.* describe four never-treated adults with classical PKU – two retarded and two with normal IQs [29]. MRS revealed high brain phe levels in the retarded and low levels in the normal individuals. Moats *et al.* carried out MRS studies in 21 classical PKU patients [30]. Four of these individuals who had high IQs, despite having high phe levels and being off diet for at least 10 years, had low brain phe. Presumably a 'modifier gene' protects the brain in these individuals.

What do these data mean? They suggest that 10% or more of patients with classical PKU (who make up ~50% of HPA patients) may not need treatment (except during pregnancy). From the Levy data, one would suspect that a larger number of individuals with atypical/mild PKU (±30%) would not need treatment (again except, perhaps, during pregnancy). MHP patients do not need treatment, even during pregnancy. Is MRS, at present, an exact, reliable and established method to sort this out? There appears to be skepticism by investigators who are unable to replicate the MRS results from the few centers who report success [31]. It appears that phe may not appear in brain MRS studies until the blood level is greater than 1200 µmol/l. Few data are available for infants and children.

However, it is obvious that, until more refined methods of determining which subjects need/do not need therapy, strict restricted-phe diet therapy must be introduced.

Diet for life

In 2001, the NIH Consensus Development Statement: phenylketonuria, unequivocally recommended the diet for life [14]. Furthermore, this document recommended blood phe levels of 120–360 µmol/l in infancy and childhood, and allowed that higher levels (<600 µmol/l) would be permissible in adolescents and adults. Recommended adolescent/adult phe levels vary in other jurisdictions: below 700 µmol/l in the UK [32], below 1200 µmol/l in Germany [33] and below 900 µmol/l in adolescence and below 1200 µmol/l in adults in France [34]. The recommendations for target levels of below 360–400 µmol/l were derived from publications by Smith and colleagues [35,36]. These were retrospective reviews and would not stand up to present-day scrutiny by the bioethics and epidemiology community, who would demand randomized, prospective, double-blind, controlled trials. Several well-designed studies reveal normal psychological and neuropsychological function in subjects with MHP (blood phe 200–600 µmol/l) on unrestricted diets [10,37]. This does not seem to fit with the lower levels recommended for infants and children on dietary treatment with the other variants (classical and atypical/mild PKU). There is no firm evidence that the higher levels 'allowed' for adolescents and adults are nontoxic. Observations in treated and untreated mild/atypical PKU (blood phe up to 900 µmol/l under 4 years of age) by Costello *et al.* revealed deficits in IQ (4.5–9 points) [38].

One suspects that at least part of the reason for the diet liberalization in this group is because most are not in 'good' control after late childhood. Most treatment clinics admit that the majority of their patients are well controlled (blood phe levels <360 µmol/l) until aged 2 or 3 years; then at least half have values above this, which becomes worse as they get older. The parents and children then get discouraged that they are not able to be compliant. Perhaps officially allowing levels up to 600 µmol/l in this group would be safe. There is some supportive evidence in animal studies whereby cerebral concentrations of phe are much higher in 4-day-old rats than in 16- or 70-day-old rats at the same blood phe levels [39]. The brutal truth is that 50–90% of adults with classical PKU are no longer on phe-restricted diets at all and, of those on diet, 70% have blood phe levels above target values [40–42].

The problem is that the restrictive, sometimes unpalatable diet is difficult to maintain, no immediate adverse effects are noted and, in fact, many adults with untreated or poorly controlled PKU function fairly well in society. Others, of course, do not, and the challenge is to identify and focus on this group and separate them from those who may not need treatment. The evidence appears to be that, after the age of 12 years, the measured IQ (e.g., Wechsler Intelligence Scale for Children [WISC-IV] or Wechsler Adult Intelligence Scale [WAIS-II]) does not usually drop significantly [43–45], although this has not yet been firmly documented. Neuropsychological (frontal lobe) function, on the other hand, shows measurable adverse changes when blood phe levels are elevated, but these reverse when blood levels are reduced [46]. Thus, in adolescents and adults, there appears to be a nonpermanent toxic effect of elevated phe on brain function. There may be more profound damage in a few. For example, Thomson *et al.* describe severe, apparently permanent, neurological damage in a small subset (0.4%) of the UK cohort [47]. The earliest treated PKU patients are now in their late 40s and early 50s. There is concern that long-term, chronic exposure to elevated phe in the brain may result in neurological and/or neuropsychological damage. This is exemplified by the anecdotal reports of severe behavioral and neurological deterioration of (untreated) institutionalized patients as they age. Three reports

of older adults with normal intellectual function and undetected PKU who, suddenly in their fourth decade, developed severe neurological deterioration, warrant concern [48–50].

Alternative methods of treatment

Difficulties with diet administration in PKU have prompted investigators to look for alternatives.

Large neutral amino acids

Oral administration of a cocktail of large neutral amino acids: tyrosine, leucine, isoleucine, valine, tryptophan, methionine and histidine, which share a common transport mechanism with phe across the blood–brain barrier (the L-type amino acid carrier) has been claimed to lower brain phe levels. This modality was first suggested as early as 1976 [51], but only anecdotal reports were available. A randomized, control study was finally reported by Schindeler *et al.*, who noted a lowering of blood phe, but were unable to confirm lower brain levels [52]. There was little difference in neuropsychological function compared with those on medical food. Problems include high cost and the large number of capsules needed to be ingested daily. Matalon *et al.* reported an open-label study, followed by a short-term, double-blind, placebo-controlled study, and claimed a lowering of blood phe by 39% [53].

Phe ammonia lyase

The phe ammonia lyase (PAL) enzyme catalyses the biotransformation of phe to a harmless metabolite, trans-cinnamic acid and traces of ammonia [54]. Use in the PKU mutant mouse has been shown to lower blood phe levels by 30–40% [55]. This may be sufficient in adolescents/adults. Problems with PAL include instability and immunogenicity. Modification with polyethylene glycol (PEG) has resulted in enhanced stability [56], and reduction of the immune response by PEGylation of surface lysine residues has shown early promise [57]. To date, all PAL studies have been performed on mice. Continuing research on designing an oral preparation for humans is ongoing.

Tetrahydrobiopterin

BH4 is a cofactor in the PAH complex and is used routinely in patients with biopterin-deficiency (malignant) PKU. Kure and associates first described BH4 use in PAH-deficiency PKU [58]. It is chiefly beneficial in patients with the milder variants (atypical/mild PKU and MHP) but is sometimes effective in cases of classical PKU with certain specific mutations. Fiege *et al.* claim 30% reductions of blood phe in 39–83% of newborns and children with MHP, 49–60% with mild/atypical PKU and 7–10% with classical PKU in 557 subjects who had BH4 loading tests [59]. A number of other investigators have reported similar results [60–63]. Detection requires genetic testing and prolonged (24–48 h) BH4-loading tests (20 mg/kg). A recent placebo-controlled study has been encouraging [64]. One problem is the cost of up to US$30,000 per year, compared with US$10,000 per year for dietary therapy. Furthermore, a combination of low-phe diet therapy plus BH4 is sometimes recommended. No studies have yet reported on the sustainability of the BH4 effect; in fact, Weglage *et al.* have reported only temporary response in some, necessitating reintroduction of dietary therapy [65]. An obvious problem is that BH4 is not effective in most patients who need it the most (classical PKU). It is now quite clear, however, that patients with MHP do not require treatment of any kind.

Gene replacement

Successful gene replacement using a viral vector has been accomplished in laboratory animals [66]. Human application has not yet been reported. A new approach, known as PAH-based targeted fusion proteins, has shown promise in laboratory studies [67].

Adequacy of current medical foods (formulae)

The nature of PKU diet therapy, synthetic medical foods and the absence of high-quality (natural) protein-containing foods has led to observations suggesting suboptimal nutritional parameters. Some of the more recent reports are discussed here.

Low bone density

Decreased bone mineralization in treated PKU has been described for at least 10 years [68–71]. Most recently, Modan-Moses *et al.* report osteopenia in 11 out of 31 (38.7%) adult PKU patients on dietary treatment and osteoporosis in two (6.5%) [72]. Various hypotheses have been explored: deficiency in protein, calcium, vitamin D trace elements or a primary defect in bone turnover, although no clear explanation has been found.

Cobalamin deficiency

Low vitamin B12 (cobalamin) levels have been reported by several authors [73,74]. One of the complications could be (permanent) subacute combined degeneration of the spinal cord. There is also the risk of hyperhomocysteinemia with various cardiovascular consequences [75]. The apparent reason is deficient intake in adolescents and young adults who neglect their (vitamin-enriched) synthetic medical food, but remain instinctively on their vegan–vegetarian diet. The possibility that high tissue levels of phe interfere with cobalamin adsorption has not been disproved.

Long-chain polyunsaturated fatty acid deficiency

Long-chain polyunsaturated fatty acids, such as arachidonic acid and docosahexaenioc acid (present only in foods from animal sources, in human breast milk and, more recently, in some infant cow's milk formulas), have been shown to be deficient in PKU-diet formulation and in PKU infants on dietary treatment [76,77]. These substances have an important role in neurotransmission and vision. Measurement of the development of visual function in infants and children has been one method of assessing the potential beneficial effects. Beblo and associates investigated visual-evoked potentials in treated patients with PKU and discovered improvement after treatment with omega-3 long-chain polyunsaturated fatty acids [78]. This same group report that fish oil supplementation enhances

n-3 long-chain omega-3 polyunsaturated fatty acids and improves motor skills [79]. Agostini *et al.* carried out a double-blind, placebo-controlled trial in 20 patients with PKU, in which long-chain polyunsaturated fatty acids were added to the phe-free medical food [80]. They demonstrated improved levels of erythrocyte docosahexaenoic acid but, 3 years later, were unable to demonstrate biochemical or functional differences.

Growth failure

Some research has claimed deficient linear growth and/or head growth in patients with PKU on dietary treatment [81], others have denied this, in fact, a number of children were overweight [82]. Published studies have suggested that this is not due to low phe [83], low tyrosine levels or deficient total protein, but may be associated with the quality of ingested protein (natural vs synthetic) [84]. In view of the data on normal neuropsychological function in MHP, an opportunity to provide more high-quality (natural) protein might be explored to allow blood phe levels of up to 600 µmol/l in infants and children, at least after 2–3 years of age and for pregnant women.

Maternal PKU: treatment prior to conception

The current recommendation for optimal treatment of MPKU is good diet control (phe 100–360 µmol/l) beginning before conception [85]. However, recent reports have shown good outcomes in pregnancies where optimal control is not obtained until as late as 8–10 weeks gestation [86]. This is reassuring to treatment centers, since numerous unexpected pregnancies (similar to the general population) occur – often despite regular and close follow-up. Congenital heart disease (up to 8% incidence), however, may not be reduced in this particular cohort. As previously stated, Levy *et al.* report that phe levels up to 600 µmol/l are safe during pregnancy [11].

Expert commentary & five-year view

What should PKU treatment centers and researchers focus on in the next 5 years?

- In my opinion, the most urgent priority is to establish adult PKU treatment centers for long-term follow-up and continuing research. These centers should be large enough (>50 patients) to assure expertise. Their mandate should include exploring treatment of institutionalized, never-treated, profoundly retarded adults;
- Since 10–20% of patients with classical PKU and 30–40% with atypical/mild PKU may not need treatment, the technology for measuring brain (vs blood) phe (MRS and other modalities) should be vigorously upgraded and pursued. This should be extended to infants and children;
- Alternative methods of treatment should continue to be actively investigated. BH4 appears to be the most promising at present but, unfortunately, seems to be ineffective in the most needy (classical PKU). The sustainability and safety of this modality must be studied;
- Fine-tuning of the semisynthetic diet needs to continue. It appears, for example, that the addition of long-chain polyunsaturated fatty acids would be safe but further observations are needed to verify efficacy;
- A sustained effort to track and recapture lost-to-follow-up adults with PKU, especially nubile females, should be a priority;
- Regular measurements of vitamin B12 and homocysteine/methylmalonic acid should be routine in adolescents and adults;
- Further studies on bone metabolism, especially long-term risk of osteopenia, should be pursued.

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Key issues

- The frequency of profound mental retardation in untreated phenylketonuria (PKU) may be less than estimated originally.
- A priority is to establish adult treatment centers for PKU and other inherited metabolic diseases.
- Non-PKU mild hyperphenylalaninemia does not need treatment, even during pregnancy.
- 'Diet for life' is unsuccessful in most adolescents and adults with PKU. Indeed, some may not need treatment. Alternative methods of treatment in this cohort must be pursued vigorously.
- Currently available dietary products may be deficient in certain nutrients.
- Blood phenylalanine levels of 200–600 µmol/l may be nontoxic, even during pregnancy.
- Treatment of maternal PKU may be successful even if initiation of therapy is delayed by 8–10 weeks.

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Affiliation

- *WB Hanley, MD, FRCPC, Professor Emeritus*
University of Toronto
The Hospital for Sick Children and the Faculty of Medicine, University of Toronto, Division of Clinical & Biochemical Genetics, Department of Paediatrics, 555 University Ave, Toronto, ON M5G 1X8, Canada
Tel.: +1 416 425 7846
Fax: +1 416 781 8515
whanley@sickkids.ca