Title: Long-term cognitive and psychosocial outcomes in adults with phenylketonuria

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ABSTRACT (259 words)

Previous studies have suggested that cognitive and psychosocial underfunctioning in early-treated adults with PKU may be explained by suboptimal adherence to dietary treatments, however these studies often employ small samples, with different outcome measures, definitions and cut-offs. Samples have also tended to comprise participants with a limited range of blood phenylalanine concentrations, and often individuals who may not have been treated early enough to avoid neurological damage. In this study, we explore the impact of lifetime dietary control, as indicated by blood phenylalanine concentrations in childhood, adolescence and adulthood, on long-term cognitive and psychosocial outcomes in a large sample of adults with PKU who were diagnosed by neonatal screening and commenced on dietary treatment within the first month of life. 154 participants underwent cognitive testing, assessing attention, learning, working memory, language, executive functioning and processing speed. 149 completed measures of psychosocial functioning, documenting educational, occupational, quality of life, emotional and social outcomes which were compared with a group of healthy controls. Many adults with PKU demonstrated cognitive impairments, most frequently affecting processing speed (23%), executive function (20%) and learning (12%). Cognitive outcomes were related to measures of historic metabolic control, but only processing speed was significantly related to phenylalanine concentration at the time of testing after controlling for historic levels. Adults with PKU did not, however, differ from controls in educational, occupational, quality of life or emotional outcomes, or on a measure of family functioning, and showed only minor differences in relationship style. These findings have implications for patient counselling and decisions regarding the management of PKU in adulthood.

SYNOPSIS

Despite being at increased risk of underperformance on tests of processing speed, executive functions and learning, adults with early-treated PKU demonstrate normal outcomes in key psychosocial domains (anxiety, depression, quality of life, educational attainment and occupational functioning). Only differences in processing speed are significantly explained by phenylalanine concentration at the time of testing.

INTRODUCTION

Phenylketonuria (PKU; OMIM 212600) is an inherited metabolic disease in which a deficiency in the enzyme phenylalanine hydroxylase (EC 1.14.16.1) impairs the conversion of the amino acid phenylalanine (phe) to tyrosine. This results in the accumulation of phe to toxic concentrations which, without treatment, results in severe neurological damage. It is the most common inherited metabolic disease in Europe and the United States with a prevalence of approximately 1:10,000.[1]

Dietary treatment of PKU was introduced in the 1950s.[2] Patients follow a protein-restricted diet and take low-phenylalanine protein substitute. Since the start of the 1970s, newborns in the UK have been screened for PKU, enabling treatment to start in the first few weeks of life and severe neurological damage to be avoided. Adhering to dietary treatment is costly and effortful[3] and many adults with PKU have phe concentrations well above current guidelines.[4] There is evidence to suggest that cognitive and psychosocial outcomes in some early-treated adults may be sub-optimal,[5,6] but the frequency of poor outcomes and how they relate to phe, particularly phe in adulthood, has yet to be fully determined.

Cognitive outcomes

Certain cognitive domains are frequently reported to be affected in early-treated adults with PKU. These include psychomotor speed,[7-9] executive functions,[7,8,11] and attention.[8,10,11] However, even in these well-studied domains, findings are not entirely consistent.[12,13]

When the relationship between these outcomes and metabolic control has been investigated, a number of studies have reported significant associations between cognitive test scores and phe during infancy and childhood, with phe concentrations earlier in life playing a greater role.[7,10,11,14] However, evidence for a relationship between outcomes and phe concentrations during adulthood is generally weaker. Significant associations have been reported with attentional abilities, although not in all studies.[6] Performance in most other cognitive domains and in psychosocial functioning has not been found to be significantly associated with adult phe.[7,12,15] Some studies have even found the reverse of the expected relationship.[9,16]

Psychosocial outcomes

There are reports of psychosocial differences between adults with PKU and controls, but existing findings are inconsistent. Some studies report that adults with PKU have lower academic attainment[17,18] and differences in occupational functioning,[19] while others do not.[20] A few studies have reported reduced quality of life in adults with PKU,[16,21,23] while others have found levels comparable with the rest of the population.[20,23] There is some suggestion that early-treated adults may be at increased risk of mood and anxiety disorders.[11]

In some studies, adults with PKU have been reported to have greater social and interpersonal difficulties[24-26] and to be less likely to achieve milestones such as marriage and parenthood than the general population.[27] However, other studies report normal outcomes in these areas.[19,20,28]

The inconsistency in the existing literature is probably at least in part due to the use of small sample sizes. Recruitment of large samples is challenging due to the small numbers of adults with PKU under follow up at individual centres, limited availability of historical phe records, and the use of long and cumbersome batteries of measures. As a result, the literature comprises numerous small samples which each use different measures and adopt different definitions and cut-offs, for example of which phe concentrations are categorised as high or low and which cognitive test scores are considered impaired. These small samples have also tended to comprise participants with well controlled blood phenylalanine,[11,13] making it difficult to fully examine the relationship between metabolic control and outcomes.

In order to try to address these issues, we describe cognitive and psychosocial outcomes and their relationships with phe concentrations in the largest sample of adults with early-treated PKU studied to date. By collecting the same set of data from a large sample, it has been possible to obtain a more accurate picture of cognitive and psychosocial outcomes and their relationship with phe concentrations throughout life. The aim of this study was to explore the impact of phe concentration in adulthood on cognitive and psychosocial outcomes once metabolic control during childhood and adolescence had been accounted for.

METHODS

The study was approved by the West Midlands – Coventry and Warwickshire NHS Research Ethics Committee and the Health Research Authority.

Participants

All eligible adults under follow-up at the Charles Dent Metabolic Unit were invited to participate. Individuals were invited to participate by post or at their routine clinic appointment and those who expressed an interest in the study received three follow up telephone reminders to complete study outcome measures. Adults who were diagnosed with PKU on newborn screening and started on treatment in the first month of life were included. No participants were receiving treatment with tetrahydrobiopterin (BH_4) which is not funded for use in the English National Health Service (NHS) except during pregnancy. Those who were late-treated or had any other condition likely to affect results were excluded (see Figure 1). 154 adults with PKU completed brief cognitive testing, and 149 completed a battery of psychosocial outcome measures. Cognitive tests were administered by or under the supervision of a clinical psychologist, in a quiet clinic room at the hospital. Psychosocial outcome measures, collected by questionnaire, were either completed at home and posted to the Metabolic Unit or completed during attendance at a routine clinic appointment.

Controls were recruited from PKU participants' families so that they would be matched as well as possible for socio-economic factors which are closely related to the outcomes under consideration.[29,30] Each participant with PKU was asked to provide one non-PKU control of similar age drawn from his or her family, ideally an unaffected sibling or another relative if no unaffected sibling was available. Controls completed an identical battery of psychosocial outcome measures. Controls did not perform cognitive tests as published normative data for the general population is available for comparison.

Measures

Metabolic control and dietary adherence

Blood phe results were obtained from medical records. The frequency of phe measurements varied over the life course of participants, as well as between participants. Other authors have sought to overcome this issue by calculating the median blood phe concentration for each six or 12 month period of life[11,31]. The same approach was adopted here. Six-monthly median phe results were then averaged to give a summary phe value known as an 'Index of Dietary Control (IDC)' for periods of early childhood (from birth up to the child's sixth birthday), late childhood (from age six to the 12th birthday), adolescence (from age 12 to the 18th birthday) and adulthood (after the 18th birthday). Both plasma and bloodspot results were included depending on availability, as there was variability in the methods used and practice had changed over the participants' lifespans. Participants who completed cognitive tests also provided a plasma sample to determine blood phe concentration on the day on which they underwent cognitive testing.

Participants who completed the battery of psychosocial questionnaires also completed a dietary questionnaire assessing self-reported adherence to a PKU diet, intake of high-protein foods and use of dietary supplements. This questionnaire is provided in the supplementary material.

Cognitive outcomes

A short battery of cognitive tests was employed focussing on attention, language, learning, working memory, executive functions and processing speed. Tests with adequate reliability

and validity and high sensitivity to the cognitive deficits that are most consistently reported in PKU were selected. All tests had high quality normative data which was stratified by age, meaning that participants' performance was compared with published normative values for their age group. The battery took 25 minutes to administer and required minimal equipment, offering good acceptability to participants and allowing data to readily be collected during a routine clinic appointment.

Attention was assessed using the forwards Digit Span task from the Wechsler Adult Intelligence Scales – Fourth Edition (WAIS-IV).[32] This test requires the participant to repeat a string of numbers which gradually increase in length, with two trials for digit strings of each length. Learning was assessed using the five trials from the California Auditory Verbal Learning Test – Second Edition (CVLT-II),[33] which requires the participant to recall a list of 16 orally-presented words. Working memory was assessed using the backwards Digit Span task from the WAIS-IV, which requires a sequence of numbers to be repeated in reverse order. As with forwards Digit Span, strings of numbers gradually increased in length, with two trials for strings of each length.

Language was assessed using semantic fluency which requires the participant to generate as many words as possible within a time limit belonging to a particular semantic category (in this case animals) within a time limit.

Executive functions were assessed a phonemic fluency task and the card sorting subtest from the Delis Kaplan Executive Function System (DKefs).[34] Phonemic fluency requires the

participant to generate as many words as possible within a time limit belonging to a particular phonemic category (in this case beginning with the letter 'S'), requiring initiation and strategy generation. In the card sorting test the participant must deduce categories in order to sort six cards into categories, and switch flexibly between different categories for sorting. Categories may be verbal, based upon words printed on the cards, for example 'animals' or 'types of transport', or perceptual, for example based upon the colour or size of cards.

Processing speed was assessed using the Symbol Digit Modalities Test (SDMT).[35] This test requires the participant to use a key to match numbers with a series of symbols, writing as many responses as possible within a time limit. This has been demonstrated to be highly sensitive to changes in psychomotor speed in other neuropsychological syndromes.[36]

Psychosocial outcomes

Psychosocial outcomes were measured with a demographic questionnaire and a range of validated self-report measures. Educational outcomes included number of years of education and highest qualification level. Occupational outcomes were socio-economic status, which was classified according to the UK Office for National Statistics simplified five-class system,[37] and the Work and Productivity Assessment Index (WPAI).[38] Quality of life was measured using the 36 Item Short Form Health Survey (SF-36).[39] SF-36 scores were collapsed into two norm-referenced T scores representing mental and physical quality of life, as per Ware and colleagues.[40] Emotional functioning was assessed using the Hospital Anxiety and Depression Scale (HADS)[41] and the Perceived Stress Scale (PSS).[42] Social functioning was measured using the Relationship Scales Questionnaire (RSQ)[43] and the

Family Adaptability and Cohesion Scale (FACES).[44] RSQ scores were used to derive scores for anxious and avoidant relationship styles following instructions from Kurdek.[45] FACES-IV scores were collapsed into a single score as per Olson and colleagues.[44]

Statistical Analyses

Data were summarized using descriptive statistics (mean and standard deviation for continuous variables and proportion for categorical variables). The distribution of our sample data was visually compared with the normal distribution using Quantile-Quantile (QQ) plots, in which the sample quantiles are plotted against the theoretical quantile (see supplementary figure). If data is normally distributed, this should result in the dots on the QQ plot falling along the diagonal line. Normality was also assessed using Shapiro-Wilk tests (see supplementary table 4). Shapiro-Wilk results for some variables reached significance, however visual examination of the plots showed that the data is close to symmetrical.

Differences between PKU group scores and published normative data were analysed by transforming raw scores into standardized z scores (by deducting the normative sample mean from each individual's actual score, and dividing the difference by the standard deviation of the normative sample). A positive z score indicates performance which is better than the normative sample average, whereas a negative z score represents performance lower than the normative sample average. z scores can be compared with standard scores, which are used to report IQ and have a mean of 100 and a standard deviation of 15; a z score of -1.5 would be equivalent to a standard (IQ) score of 77.5. One-sample t-tests were then used to compare mean z scores for the PKU group with the expected population mean of 0. Scores were

categorised as impaired if they fell at or below 1.5 standard deviations below the normative sample mean. Such a cut-off is commonly adopted in neuropsychological research.[46] 6.68% of scores from the normal population would be expected to fall in this range on any one test.

Differences between PKU and control groups, or between subgroups of PKU participants, were analysed using Multivariate Analysis of Variance (MANOVA). Comparisons of categorical outcomes were made using Chi-square tests with appropriate continuity correction (Yates correction), or Fisher's exact test where expected cell values were small. As a large number of psychosocial domains were assessed, five key psychosocial outcomes were prioritised for comparison: anxiety, depression, quality of life, educational attainment and occupational functioning.

The contribution of phe to the variance in cognitive outcomes was modelled using linear regression, in order to examine the impact of phe concentration at each stage of life when all other stages were controlled for. Age and sex were entered into the model in order to control for these variables. Indices of Dietary Control (IDCs) during early childhood, late childhood, adolescence, and adulthood were included as independent variables, as well as plasma phe concentration on the day of cognitive testing. z scores on each cognitive test were dependent variables. Multicollinearity was assessed using Variance Inflation Factors (VIFs) and no pair of independent variables was found to have a VIF value larger than 10, indicating an acceptable level of collinearity.

Based on the existing literature, subgroups were created for comparisons which could help determine the importance of metabolic control in adulthood. First, PKU participants were grouped according to their self-reported adherence to a PKU diet. Participants classified their adherence as either on-diet (taking supplements and restricting natural protein), off-diet (neither taking supplements nor restricting dietary protein) or partially-adherent (either taking supplements or restricting protein). Secondly, participants were classified by their objective phe concentrations during childhood and adulthood. Jahja and colleagues[11] examined the difference in outcome between 11 adults with PKU who maintained IDCs <360 umol/L before their 12th birthday but had IDCs ≥360 umol/L after 12 years (low-high group) and seven adults whose IDCs both before and after age 12 were ≥360 umol/L (high-high group). Their sample size did not permit statistical comparisons with those who had IDCs <360 before their 12th birthday and <360 after 12 years, a 'low-low' group, which is critical for evaluating the impact of dietary relaxation later in life. In order to allow the creation of adequately sized groups and better reflect historical phe target concentrations in the UK, we created a low-low group who had IDCs <600 umol/L before age 12 and also after age 18, and a low-high group with IDCs <600 umol/L before age 12 but ≥1000 umol/L in adulthood. A third, high-high group had IDCs ≥600 umol/L before age 12 and ≥1000 umol/L after age 18.

Sensitivity analyses

Due to a lower response rate on psychosocial outcome measures amongst controls than in the PKU group, a matched control was not recruited for every participant with PKU. In order to make use of all of the data collected, unpaired analyses are reported below. However, this means that not all observations are equally independent, as some are collected from related pairs (i.e. siblings) while others are not. In order to assess the impact on the results, equivalent paired analyses were also run using only those data sets where a matched PKU-

control pair was available (using the paired Hotelling t^2 test for continuous variables and McNemar's test for categorical outcomes).

The validity of neuropsychological test scores can be influenced by the degree of effort exerted by the subject. An embedded measure known as *Reliable Digit Span* (RDS) can be computed by summing the longest forward and backward Digit Span items completed correctly on both trials, and has been demonstrated to provide a useful screen for low effort, with a cut-off score of seven providing good sensitivity and specificity.[47] Over 90% of PKU participants obtained RDS scores above the cut-off. As an additional sensitivity analysis, analyses involving cognitive test results were repeated after excluding those participants who failed the embedded effort test.

Missing data

Missing data arose via three mechanisms:

- Patients who met the inclusion criteria but did not respond to invitations to participate or declined to participate (N = 152, see Figure 1).
- Questionnaire items that were missed (148/3749 scores missing, 3.9%).
- Cognitive test batteries that were not completed (16/1078 scores missing, 1.5%).
- Historical medical records that were unavailable, for example childhood phenylalanine concentrations were not available for all participants who completed cognitive outcome measures (80 participants did not have IDCs available for all time periods – early childhood, late childhood, adolescence and adulthood).

The sensitivity of the findings to this missing data was assessed on the basis of imputation of missing data using linear regression. A regression model is used to create a prediction of every patient's outcomes on the basis of routinely-collected clinical data including age, sex, years of education and historic and current phe concentrations. The difference between a patient's true and predicted score on any outcome is known as a residual, and the distribution of residuals can be calculated for any particular regression model. For individuals with missing data, missing scores were predicted by the regression model and a randomly drawn residual was added to each predicted score. The process was repeated until the imputed model reached stability. Imputed and non-missing data were then combined and analysed using the methods described above. For further information about this technique, see Howell, 2007.[48] Imputed data was used only for the purpose of sensitivity analysis and no imputed data is included in the results reported below.

Analyses were carried out using SPSS version 25 and R version 3.6.2.

RESULTS

PKU and control group demographics are summarized in Table 1. There were no significant differences in age ($t(223) = 1.860$, p-value = 0.064), sex ($\chi^2(1) = 0.000$, p-value = 0.986) or years of education ($t(221) = -0.438$, p-value = 0.661) between controls and adults with PKU who completed the psychosocial questionnaires. No participants were using tetrahydrobiopterin (BH_4) treatment because this is not funded for the routine treatment of adults in the NHS.

Metabolic control and dietary adherence

Metabolic control is summarised in Table 1. As shown, participants demonstrated a wide range of IDCs in adulthood (above age 18), from below 200 to over 1800 umol/L. This allows the relationship between metabolic control and outcomes to be examined in PKU adults with a range of blood phe concentrations.

76 of the participants who completed the diet questionnaire classified themselves as on diet while 47 were off diet and 18 were partially adherent. Of 74 patients with sufficient historical phe data, 27 met the criteria for the low-low phe group, having IDCs <600 umol/L before age 12 and also after age 18. 38 met criteria for the low-high group with IDCs <600 umol/L before age 12 but ≥1000 umol/L in adulthood. Only nine could be categorised as high-high, with IDCs of ≥600 umol/L before age 12 and ≥1000 umol/L after age 18 years.

Cognitive outcomes

Cognitive test scores are summarised in Table 2 and their distributions are shown in Figures 2a-g. Results for the Shapiro-Wilk tests for statistical normality are provided in supplementary table 4. The scores for attention, working memory and executive function (DKefs card sorting) do not follow the normal distribution.

Attention, learning and working memory

Compared to the normative sample, the PKU group had significantly lower forwards ($t(152) = -4.015$, p-value < 0.001; Figure 2c) and backwards digit span z scores ($t(152) = -6.956$, p-value < 0.001; Figure 2d). There was no significant group difference in mean CVLT-II z

scores (t(151) = -0.220, p-value = 0.826; Figure 2b) although 12% of participants had impaired scores compared with 6.68% in published normative data.

Executive function

The PKU group underperformed on the 'S' test of phonemic fluency, with a mean z score of -0.44 (t(149) = -5.537, p < 0.001; see Figure 2e). 13% of scores were impaired. Semantic fluency scores did not differ significantly from published normative data (t(149) = 0.902, p = 0.369; Figure 2f). Card sorting was less impaired with a mean z score of -0.26, however this is still statistically lower than the normative sample (t(151) = -3.137, p-value <0.01; see Figure 2g). Only 9% of card sorting scores were impaired, which is close to the 6.68% of impaired scores expected in the general population.

Processing speed

The most marked cognitive difference emerged on the SDMT test of processing speed. Mean z score was -0.61, making the difference from the published normative data highly significant (t(151) = -5.859, p-value < 0.001) and 23% (35/149) of scores were impaired (Figure 2a).

Overall, 53% of the sample had no impaired scores on any of the cognitive tests and 47% had an impaired score on at least one test. Further detail on the number of impaired cognitive test scores obtained by adults with PKU can be seen in the supplementary material (Supplementary table 2). Whilst only 6.68% of the normal population would be expected to obtain an impaired score on any individual test, approximately 31% of the normal population

would be expected to obtain at least one impaired score on this particular battery of tests, because the probability of obtaining one or more impaired scores increases with the number of tests administered and the degree to which they are correlated.[49] See the supplementary material for observed Pearson product moment correlations between scores on each cognitive test (Supplementary table 3).

Differences between PKU subgroups

On-diet, off-diet and partially-adherent groups did not differ on cognitive outcomes (Hotelling's $t^2 = 0.148$, $F(21, 491) = 1.156$, p-value = 0.286). There was no significant difference between adults who maintained IDCs <600 umol/L before age 12 and also after age 18 (low-low group) and those whose phe concentrations were <600 umol/L before age 12 but >1000 umol/L after age 18 (low-high group) on any of the cognitive measures (Hotelling's $t^2 = 0.104$, $F(14,330) = 1.225$, p-value = 0.255). Descriptive statistics for cognitive test scores in each of the three groups are included in the supplementary material (Supplementary table 1).

Relationship of cognitive scores with phenylalanine concentrations

53% (79/147) of adults in our sample had normal scores on all seven tests. Phe at the time of testing ranged from 119-2135 umol/L in adults who did not have any impaired scores on cognitive tests. 78% (60/77) of those whose plasma phe concentration was measured at the time of testing with a result above 600 umol/L had no impaired scores on any cognitive tests. Thus meeting recommended target phe concentrations[50] did not appear to be necessary for normal performance on cognitive tasks.

Blood phe concentrations accounted for a significant amount of variance in two cognitive domains: processing speed (measured on the SDMT; see Figure 3-A) and learning (measured on the CVLT-II; see Figure 3-B). Processing speed was significantly correlated with average phe concentrations in adulthood (after age 18) and with plasma phe concentration on the day of cognitive testing, with higher phe concentrations being associated with slower performance. However, once phe concentrations at all other stages (including early childhood, late childhood and adolescence) were controlled for, only phe concentrations in early childhood (β = -0.00299, SE(β) = 0.00122, t(64). = -2.45, p-value = 0.0172) and on the day of testing (β = -0.00105, SE(β) = 0.000476, t(64). = -2.20, p-value = 0.0311) could explain a significant amount of variance in speed (Supplementary table 5). The beta coefficient of -0.00105 suggests that an increase in phenylalanine concentration of 1000 umol/L (on the day of testing) would be associated with a decrease in processing speed of -1.05 standard deviations. Learning scores were also significantly negatively correlated with average phe concentrations in adulthood and with plasma phe concentration on the day of testing. However, once phe concentrations at all other stages were controlled for, only phe concentrations during late childhood (age six to 12) were independently associated with performance as an adult (β = -0.00278, SE(β) = 0.00120, t(64). = -2.32, p-value = 0.0236).

Psychosocial outcomes

Demographic and psychosocial outcomes are summarised for continuous variables in Table 3 and categorical variables in Table 4. Categorical findings are described below while group differences in continuous variables are assessed in the between-group MANOVA.

Educational attainment

The proportion of adults achieving each qualification level did not differ between the PKU group and controls (p-value = 0.951; see Table 4). Over 35% of adults with PKU obtained a university degree and nearly 20% completed postgraduate qualifications.

Occupational functioning

The proportion of participants in each employment type (full-time, part-time etc.) did not differ between the PKU and control groups (p-value = 0.250). Self-reported work capability on the WPAI also did not differ (p-value = 0.944), with 91% of adults with PKU reporting completely normal work capability. There was no difference in socio-economic status (p-value = 0.282). 37% of the PKU group fell in the highest category of SES, representing managerial, administrative or professional occupations.

Emotional functioning

The proportion of individuals falling into normal, mild, moderate and severe ranges for anxiety (p-value = 0.172) and depression (p-value = 0.164) on the HADS did not differ significantly between PKU and control groups.

Social functioning

Relationship status did not differ between the two groups (p-value = 0.509).

Adults with PKU were not significantly less likely to have children than controls (41% versus 51%, $\chi^2(1) = 1.576$, p-value = 0.209) with a similar average number of children per participant (0.84 versus 0.71; t(221) = 0.954, p-value = 0.341).

Differences between groups

The MANOVA comparing psychosocial outcomes between adults with PKU and controls revealed that years of education and scores for stress, anxiety, depression, mental quality of

life, physical quality of life, avoidant relationship style, and family functioning did not differ from controls. However, on average individuals with PKU had a more anxious relationship style than controls (Hotelling's $t^2 = 0.107$, $F(8, 174) = 2.317$, p-value = 0.022; Fisher's protected $t(1,211) = -2.274$, p-value = 0.024).

Differences between PKU subgroups

A significant difference in psychosocial outcomes did emerge between the dietary adherence subgroups (Hotelling's $t^2 = 0.134$, $F(12, 659) = 2.453$, p-value = 0.004). Examination of between-subjects effects indicated that significant differences occurred in mental and physical quality of life. Post-hoc tests using Tukey's Honest Significant Difference test showed that the partially-adherent group obtained significantly lower mental and physical quality of life scores than the adherent and non-adherent groups. The on-diet group and off-diet group did not differ in key psychosocial outcomes.

Low-low and low-high phe subgroups did not differ significantly in key psychosocial outcomes (Hotelling's $t^2 = 0.056$, $F(8, 442) = 1.554$, p-value = 0.136). The high-high group was not large enough to permit significance testing, however it is interesting that in these patients mean scores for anxiety appeared to be lower (high-high = 5.00, low-low = 9.07, low-high = 10.29), relationship style appeared to be less anxious (high-high = 1.80, low-low = 2.27, low-high = 2.40) and mental quality of life appeared to be better (high-high = 47.47, low-low = 38.87, low-high = 34.67) than in patients who achieved the lowest average phe concentrations during childhood. Thus emotional and social outcomes appear to be poorer in

adults with PKU who maintained lower blood phe concentrations as children, although it was not possible to test the significance of this difference.

When the analyses were then carried out using only paired data, or with only participants who passed the test of effort, or including imputed data where values were missing, then this did not change these findings, indicating that the findings are robust to sensitivity analyses and the inclusion of imputed missing data.

DISCUSSION

Our large sample of adults with PKU did show some differences from population norms on cognitive tests, which are consistent with other published studies.[7,9] More than half of adults with PKU, however, obtained scores within the normal range in all domains tested. In addition, adults with early-treated PKU had normal educational and occupational outcomes, and rates of clinical anxiety and depression which did not differ from matched controls. For those who achieved adequate metabolic control during childhood, there was no difference in key cognitive and psychosocial outcomes between those who continued to maintain low blood phe concentrations as adults and those who considerably relaxed their dietary treatment.

Cognitive outcomes

On cognitive testing, the distribution of scores seen in adults with PKU is shifted on some tests such that a greater number of subjects have impaired scores (<1.5 SD below the mean of published normative data; see Figure 2). This is most pronounced for the Symbol Digit Modalities Test, a test of processing speed. Processing speed is the cognitive domain most

strongly associated with subcortical white matter changes, which have been reported in PKU.[5,51] Impaired scores were also more frequent than expected on certain tests of executive function, working memory and attention, in keeping with the findings of others.[8,10,11]

Metabolic control in childhood is known to be critical in ensuring good cognitive outcomes in adulthood;[52] however historically it has been difficult to untangle the role for phe concentration during development from phe during adulthood. This large sample allows us to control for concentrations during critical periods in childhood and adolescence when analysing the impact of phe on adult cognitive function.

Phe at the time of cognitive testing was only found to make a unique contribution to variance in one domain, processing speed. Phe at the time of testing made no significant contribution to scores on tests of executive function, attention, learning or working memory once historical concentrations had been controlled for. Some adults with low current phe concentrations demonstrate deficits and other adults with very high phe concentrations show normal cognitive test results, as has also been reported by Romani and colleagues.[53] Therefore it seems that current dietary adherence has the potential to exert a small impact on processing speed in some adults with PKU, although there is significant inter-individual variability. Conversely it appears that dietary control alone is not sufficient to prevent all suboptimal cognitive outcomes. This may help inform decisions surrounding continuance or return to diet, however given that lower quality of life is associated with partial adherence to a PKU diet, this should be an individualised decision. Such inter-individual variability in outcomes which cannot be accounted for by phe concentrations has been observed by others.[54] Future research should try to elucidate the factors which may explain this variability.

Psychosocial outcomes

Our data indicate that adults with early-treated PKU demonstrate positive long-term psychosocial outcomes in the domains of education, occupational functioning, quality of life and emotional and social functioning. This confirms the success of newborn screening and early treatment for PKU. The parents of a baby newly-diagnosed with PKU can be reassured that with good metabolic control during early life (we have used up to the age of 12 in our analysis), their child should grow up to have psychosocial outcomes comparable with the general population. These positive outcomes are in keeping with some smaller studies, for example Bosch and colleagues[20] (2009) and Cazzorla and colleagues.[23] Our findings differ from other samples, notably the Dutch sample described in detail by Huijbregts and colleagues,[22] who reported reduced quality of life in 58 adults with PKU. This could reflect a variety of factors, such as differences between the Dutch and British PKU populations or healthcare systems, the different measures used, or the tighter metabolic control and lower blood phe concentrations seen in the Dutch sample, which could be associated with a negative impact on quality of life.

Adults with PKU reported a more anxious relationship style than their relatives. Relationship styles are believed to be influenced by a child's relationship with their caregivers during development, and therefore might be expected to be impacted by the diagnosis of a long-term condition and by its management with a difficult and demanding dietary treatment. Carpenter and colleagues[55] have proposed a model for the relationship between parental adjustment and metabolic control. Increased parental anxiety and anxious control of dietary treatment could result in lower phe concentrations during childhood, but also in a more anxious relationship

style. Interestingly, higher levels of anxiety were observed in those who had the lowest blood phe concentrations as children, as has also been reported by Palermo and colleagues.[56] Clinicians in paediatric centres should be sensitive to the potential impact of the families' psychological adjustment to PKU on their child, and consider offering psychological support at this stage.

The stresses involved in following a PKU diet can also affect adults with PKU. We found that self-reported partial adherence to a PKU diet was associated with significantly poorer quality of life than that seen both in those who were confident with their dietary management, and in those who were on unrestricted diets. Individuals who are struggling with their dietary management require additional support, either to help build confidence in managing a PKU diet (which may require individualised goals), or to support them to come off the diet altogether if that is their informed decision. This study shows that discontinuation of a PKU diet in adulthood in no way precludes excellent psychosocial and cognitive functioning.

There are some limitations to these findings. A brief battery of cognitive tests was employed, which allowed collection of data from a large number of participants but only across a small number of domains of particular interest. We attempted to control statistically for effects of sex and age by including these variables in the regression analyses, however the effects of phe concentrations in different age groups should be interpreted with caution. In order to draw stronger conclusions about the causal relationship between metabolic control and long-term outcomes we will need longitudinal data. However, this paper has provided a larger sample than any previous studies of long-term outcomes in adults with PKU, covering a wide range of levels of metabolic control. This has made it possible to evaluate the relationship

between current and average phe concentrations in adulthood and outcomes, while controlling for phe in childhood and adolescence.

We have described cognitive and psychosocial outcomes from the largest sample of adults with early-treated PKU to date and been able to examine the relationship between these outcomes and historical metabolic control in a significant proportion. The findings confirm previous reports of excellent quality of life, academic attainment and occupational functioning, despite the presence of a higher than expected rate of impaired cognitive test scores. We found lower rates of mood and anxiety disorder than those previously reported in samples from the Netherlands, perhaps because this is a larger sample with a wider range of metabolic control. The findings help in counselling adults with early-treated PKU about their dietary choices; there is evidence that in some adults lower phe concentrations can be associated with slightly improved processing speed, but excellent functioning and quality of life are possible without adhering to a strict diet. Currently, the long-term implications of raised phenylalanine for old age remain unknown.

Importantly, our data emphasise the need for a flexible treatment approach for adults with PKU which gives emphasis to both psychosocial and cognitive functioning, acknowledges the potential adverse effects of partially adhering to a PKU diet and recognises our patients' personal preferences and individualised goals.

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FIGURES AND TABLES

Table 1: Descriptive statistics for demographic and metabolic control outcomes in PKU and control participants

	PKU Group				**Control Group**	
	Cognitive test group (Total N=154)	**Number for whom results were obtained**	**Questionnaire group (Total N=154)**	**Number for whom results were obtained**	**Total N=76**	**Number for whom results were obtained**
Mean age (SD) years	32.38 (9.04)	154	33.93 (8.90)	149	36.29 (9.15)	76
Male / Female	69 / 85	154	59 / 90	149	30 / 46	76
Mean years of education (SD)	14.90 (13.11)	145	15.42 (3.32)	148	15.21 (3.29)	75
Mean IDC[1] 0-6 years (SD,	404 (153, 153-1051)	88	425 (159, 208-1051)	75	-	-

range) umol/L)						
Mean IDC[1] 6-12 years (SD, range) umol/L)	549 (208, 232-1185)	91	569 (210, 230-1185)	76	-	-
Mean IDC[1] 12-18 years (SD, range) umol/L)	739 (248, 248-1438)	99	731 (257, 201-1387)	88	-	-
Mean IDC[1] >18 years (SD, range) umol/L)	908 (359, 168-1869)	147	837 (327, 185-1770)	143	-	-

[1]Index of Dietary Control (IDC)

Table 2: Descriptive statistics for cognitive outcomes in adults with PKU

Domain	**Test**	**N**	**Mean z score (SD)**	**% Impaired**[1]
Attention	WAIS-IV Digits Forwards	153	-0.33 (1.00)***	7.69
Learning	CVLT-II Trials 1-5	152	-0.02 (1.22)	11.61
Working memory	WAIS-IV Digits Backwards	153	-0.47 (0.83)***	6.67
Language	Animals	150	0.07 (0.99)	3.40
Executive function	S	150	-0.44 (0.97)***	12.93
	DKefs Card Sorting	152	-0.26 (1.00)**	9.39
Processing speed	SDMT	152	-0.61 (1.29)***	23.49

[1]Defined as a score falling at or below 1.5 standard deviations below the normative sample mean. 6.68% of scores from the normal population would be expected to fall in this range on any one test.

significantly different from normative sample mean at $p<0.01$; * significantly different from normative sample mean at $p<0.001$

Table 3: Descriptive statistics for continuous psychosocial outcomes in adults with PKU and control participants

Measure	**PKU Group**		**Control Group**	
	N	**Mean (SD)**	**N**	**Mean (SD)**
SF-36 Mental Quality of Life	149	42.36 (13.67)	74	45.60 (13.45)
SF-36 Physical Quality of Life	149	50.67 (8.70)	74	50.73 (8.06)
Perceived Stress Scale	149	16.14 (7.39)	76	14.73 (6.62)
HADS[1] Anxiety	147	7.43 (4.82)	75	6.80 (3.72)
HADS Depression	147	4.15 (3.64)	75	3.72 (3.59)
WPAI[2] Work Productivity	115	1.32 (1.96)*	53	0.57 (1.22)*
RSQ[3] Anxious	139	2.17 (1.02)*	74	1.87 (0.84)*
RSQ Avoidant	139	2.52 (0.49)	74	2.62 (0.49)
FACES-IV[4] Total Ratio	124	1.92 (0.64)	72	1.88 (0.58)

[1]Hospital Anxiety and Depression Scale

[2]Work Productivity and Activity Impairment Questionnaire

[3]Relationship Styles Questionnaire

[4]Family Adaptability and Cohesion Scale

*significantly different at $p<0.05$

Table 4: Descriptive statistics for categorical psychosocial outcomes in adults with PKU and control participants

Domain	**Classification**	**PKU Group N (%)**	**Control Group N (%)**
Qualification level	None	6 (4.1)	2 (2.6)
	GCSE[1]	25 (16.9)	14 (18.4)
	Apprenticeship	3 (2.0)	1 (1.3)
	A-level[2]	33 (22.3)	17 (22.4)
	Degree	53 (35.8)	32 (42.1)
	Postgraduate	28 (18.9)	10 (13.1)
Socio-economic status	1	55 (37.4)	29 (38.6)
	2	49 (33.3)	24 (32.0)
	3	28 (19.0)	9 (12.0)
	4	7 (4.8)	9 (12.0)
	5	8 (5.4)	4 (5.3)
Employment type	Full-time permanent	84 (57.1)	42 (55.3)
	Full-time temporary	5 (3.4)	2 (2.6)
	Part-time permanent	28 (19.0)	9 (11.8)

	Casual	4 (2.7)	6 (7.9)
	Other	26 (17.7)	17 (22.4)
Work capability	Normal	126 (91.3)	61 (89.7)
	Normal hours, reduced capability	4 (2.9)	2 (2.9)
	Adjusted hours or duties	5 (3.6)	3 (4.4)
	Absent with slight restriction of ADLs[3]	3 (1.5)	2 (2.9)
	Absent with major restriction of ADLs	1 (0.7)	0 (0.0)
Relationship status	Married/Civil partnership	59 (40.1)	40 (52.6)
	Cohabiting	30 (20.4)	13 (17.1)
	Never married	51 (34.7)	21 (27.6)
	Divorced	5 (3.4)	1 (1.3)
	Widowed	0 (0.0)	0 (0.0)
	Separated	2 (1.4)	1 (1.3)

[1]GCSE: General Certificate of Secondary Education (age 16)

[2]Advanced-level Qualification (age 18)

[3]Activities of Daily Living

Supplementary table 1: Descriptive statistics for cognitive outcomes in PKU subgroups

Domain	**Test**	**Low-low group mean z score (SD)**	**N**	**Low-high group mean z scores (SD)**	**N**	**High-high group mean z score (SD)**	**N**
Attention	WAIS-IV Digits Forwards	-0.40 (0.81)	15	-0.23 (1.08)	28	-0.12 (1.12)	9
Learning	CVLT-II Trials 1-5	-0.22 (1.39)	15	-0.30 (1.25)	28	-0.80 (0.84)	9
Working memory	WAIS-IV Digits Backwards	-0.63 (0.88)	15	-0.50 (0.93)	28	-0.74 (0.91)	9
Language	Animals	-0.17 (1.24)	15	-0.10 (1.03)	27	0.30 (1.60)	9
Executive function	S	-0.33 (1.19)	15	-0.37 (0.96)	27	-0.34 (1.18)	9
	DKefs Card Sorting	-0.09 (0.73)	15	-0.42 (0.87)	28	-0.39 (0.55)	9

Processing speed	SDMT	-0.44 (1.54)	15	-1.19 (1.12)	28	-0.98 (1.50)	9

Supplementary table 2: Frequency of impaired cognitive test scores (at or below 1.5 standard deviations below the normative sample mean) in adults with PKU

Number of impaired scores	**N (%) of PKU group**
0	79 (53.7)
1	41 (27.9)
2	15 (10.2)
3	9 (6.1)
4	3 (2.0)
5	0 (0)
6	0 (0)
7	0 (0)

Supplementary table 3: Correlations between neuropsychological test scores in adults with PKU

	WAIS-IV Digits Forwards	**CVLT-II Trials 1-5**	**WAIS-IV Digits Backwards**	**Animals**	**S**	**Card Sorting**	**SDMT**
WAIS-IV Digits Forwards	1	0.181*	0.448**	0.218**	0.367**	0.221**	0.238**
CVLT-II Trials 1-5		1	0.292**	0.344**	0.249**	0.392**	0.315**
WAIS-IV Digits Backwards			1	0.224**	0.208**	0.238**	0.318**
Animals				1	0.387**	0.170*	0.348**
S					1	0.195*	0.254**
Card Sorting						1	0.176*
SDMT							1

*significant at p<0.05 two-tailed; **significant at p<0.01 two-tailed

Supplementary table 4: Shapiro-Wilk Test for cognitive outcomes

Domain	**Test**	**W**	**p-value**
Attention	WAIS-IV Digits Forwards	0.96	<0.01
Learning	CVLT-II Trials 1-5	0.99	0.57
Working memory	WAIS-IV Digits Backwards	0.95	<0.01
Language	Animals	0.99	0.74
Executive function	S	0.99	0.76
	DKefs Card Sorting	0.83	<0.01
Processing speed	SDMT	0.99	0.63

Supplementary table 5: Results of multiple regression analyses

Domain (test)	**Mean IDC**	**Beta coefficient (standard level of beta)**	**T (p-value)**
Attention (Digits Forwards)	0-6 years	0.00159 (0.00107)	1.485 (0.142)
	6-12 years	-0.00141 (0.00103)	-1.367 (0.177)
	12-18 years	0.00160 (0.000931)	1.716 (0.0910)
	>18 years	-0.000947 (0.000650)	-1.457 (0.150)
	Day of testing	0.000181 (0.000418)	0.432 (0.667)
Learning (CVLT-II)	0-6 years	-0.00117 (0.00124)	-0.945 (0.348)
	6-12 years	-0.00277 (0.00120)	-2.320 (0.0236)*
	12-18 years	0.00102 (0.00108)	0.949 (0.346)
	>18 years	-0.000307 (0.000483)	-0.409 (0.684)
	Day of testing	-0.000788 (0.000483)	-1.629 (0.108)
Working memory (Digits Backwards)	0-6 years	$3.139e^{-04}$ ($8.603e^{-04}$)	0.365 (0.716)
	6-12 years	$-1.018e^{-03}$ ($8.306e^{-04}$)	-1.225 (0.225)
	12-18 years	$1.45e^{-04}$ (8.306e-04)	0.194 (0.847)
	>18 years	$-7.439e^{-05}$ ($5.221e^{-04}$)	0.194 (0.847)
	Day of testing	$-9.774e^{-05}$ ($3.358e^{-04}$)	-0.142 (0.887)
Executive function (S)	0-6 years	0.00118 (0.00112)	1.055 (0.295)

	6-12 years	-0.000859 (0.00108)	-0.795 (0.429)
	12-18 years	0.000134 (0.00100)	0.133 (0.984)
	>18 years	0.000210 (0.000689)	0.305 (0.762)
	Day of testing	-0.000244 (0.000437)	-0.558 (0.579)
Executive function (Card Sorting)	0-6 years	$2.764e^{-05}$ ($1.154e^{-03}$)	0.024 (0.981)
	6-12 years	$-8.863e^{-04}$ ($1.114e^{-03}$)	-0.796 (0.429)
	12-18 years	$-4.526e^{-04}$ ($1.003e^{-03}$)	-0.451 (0.653)
	>18 years	$-1.435e^{-05}$ ($7.001e^{-04}$)	-0.020 (0.984)
	Day of testing	$-3.105e^{-04}$ ($4.504e^{-04}$)	-0.689 (0.493)
Processing speed (SDMT)	0-6 years	-0.00299 (0.00122)	-2.45 (0.017)*
	6-12 years	-0.00154 (0.00118)	-1.31 (0.196)
	12-18 years	0.00167 (0.00106)	1.57 (0.122)
	>18 years	-0.000771 (0.000741)	-1.041 (0.0311)
	Day of testing	-0.00105 (0.000477)	-2.204 (0.0311)*

*$p<0.05$

Figure legends

Figure 1: Eligibility and recruitment (Consort Flow Diagram)

Figures 2a-g: The distribution of z scores on each cognitive test for the PKU group are shown in solid blue. These can be compared with the expected distribution in the normal population, shown in dashed pink. Mean PKU z scores are significantly different from expectation on all but two of the tests (verbal learning and semantic fluency).

Figure 3-A (a-e): Standardised processing speed scores (SDMT) are plotted against phe concentration (mean IDC) at various stages of life. The blue line represents linear fit – the linear association between z scores and phe concentrations – which has a 95% probability of falling within the shaded grey area. Psychomotor speed was significantly correlated with average phe concentrations in adulthood (d)($r = -0.23$, $p = 0.005$) and with plasma phe concentration on the day of testing (e)($r = -0.26$, $p = 0.012$), with higher phe concentrations being associated with slower performance.

However, once phe concentrations at all other stages were controlled for, only phe concentrations in early childhood (age 0-6 years)($\beta = -0.00299$, $SE(\beta) = 0.00122$, $t(64). = -2.45$, p-value = 0.0172) and on the day of testing ($\beta = -0.00105$, $SE(\beta) = 0.000476$, $t(64). = -2.20$, p-value = 0.0311) could explain a significant amount of the variance in speed (Supplementary table 5).

Results at other ages included (a) Phe at 0-6 years; $r = -0.11$, $p = 0.299$; (b) Phe at 6-12 years; $r = -0.02$, $p = 0.889$ and (c) Phe at 12-18 years; $r = -0.07$, $p = 0.516$.

Figure 3-B (f-j): Learning (CVLT-II Trials 1-5) scores are plotted against phe concentration (mean IDC) at various stages of life. The blue line represents linear fit – the linear association between z scores and phe concentrations – which has a 95% probability of falling within the shaded grey area. Learning was significantly correlated with average phe concentrations in adulthood (i) ($r = -0.193$, $p = 0.020$) and with plasma phe concentration on the day of testing (j) ($r = -0.256$, $p = 0.002$), with higher phe concentrations being associated with poorer performance.

However, once phe concentrations at all other stages were controlled for, only phe concentrations during late childhood (age 6-12 years) were independently associated with performance as an adult (β = -0.00278, SE(β) = 0.00120, t(64). = -2.32, p-value = 0.0236)(Supplementary table 5).

Results at other ages included (f) Phe at 0-6 years; r = -0.10, p = 0.352; (g) Phe at 6-12 years; r = -0.15, p = 0.148 and (h) Phe at 12-18 years; r = -0.153, p = 0.133.

Supplementary figure: QQ plots for cognitive outcomes

Demographic questionnaire (for supplementary material)

Investigating outcomes in adults with early-treated phenylketonuria
Demographics Questionnaire

Name:

Contact telephone number:

Date of birth:

Country of birth:

First language:

Other languages:

At what age did you leave education?

Please select the highest level of qualifications that you hold (tick the box). Please also write the number of these qualifications that you hold in the space provided.

A. None
B. O-level/GCSE How many?
C. Apprenticeship
D. A-level/equivalent How many?
E. Degree
F. Postgraduate

What is your current job title?

How long have you worked in this job?

How many hours do you normally work per week in your job?

How many hours overtime do you work in your job in an average week?

How many hours per week do you work on any other job? (mark "0" if no other job)

What do you earn from your work per month? £

How much do you receive in state benefits payments per month (e.g. Employment and Support Allowance, Jobseekers Allowance, Universal Credit)? £

What is your total household income per month? £

Which of these options best describes your current work situation?

A. Full-time permanent employee
B. Full-time temporary employee
C. Part-time permanent employee
D. Casual
E. Other

Which of these options best describes your current work?

A. Working normally with no reduced capabilities
B. Working normal hours with reduced capability
C. Working adjusted duties (hours or activities)
D. Absent from work with slight restriction of daily living
E. Absent from work with major restriction of activities of daily living

What is your marital status?

A. Married or civil partnership
B. Cohabiting
C. Single, never married or in a civil partnership
D. Single, divorced
E. Single, widowed
F. Separated, still legally married/civil partnership

If you have children living at home, how many are in each of the following age groups:

A. Less than 4 years old

B. 4 through 12 years old

C. 13 through 18 years old

D. 19 and over

Thank you for taking the time to complete this questionnaire

Investigating outcomes in adults with early-treated phenylketonuria
Diet Questionnaire

Name:

Date of birth:

Your diet in the past:

At what age did you discontinue PKU dietary treatment in childhood (if you have not discontinued diet, write 'never'): years

If you stopped following a PKU diet in childhood, have you ever returned to it as an adult (not including times when you were following a preconception or pregnancy diet)?

A. Yes
B. No

How old were you when you re-started diet? years

If you have returned to diet as an adult, are you still following a PKU diet?

A. Yes
B. No

If not, how long did you spend back on diet? years/months
(please circle as appropriate)

Have you tried to return to diet on more than one occasion?

A. Yes
B. No

Your current diet:

Which of the following best describes your diet?

A. I am on a PKU diet with restricted protein and PKU supplements
B. I continue to take supplements but not to restrict protein
C. I continue to limit my dietary protein intake but do not take PKU supplements
D. I am completely off diet

If you currently restrict the protein in your diet, how many exchanges or grams of protein do you consume per day? exchanges

If you currently take PKU supplements, how many do you take per day? supplements per day

How often, on average, do you eat each of the following foods? Place a tick in the relevant column.

Food	**Average use**							
	never	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	3+ per day
Meat								
Fish/seafood								
Eggs								
Dairy								

If you are not currently following a PKU diet, do you take any vitamin and mineral supplements regularly because of PKU?

A. No – no supplements
B. Yes – multivitamin & mineral supplement
C. Yes – vitamin B12 tablets
D. Yes – vitamin B12 injections

Thank you for taking the time to complete this questionnaire

Figure 1

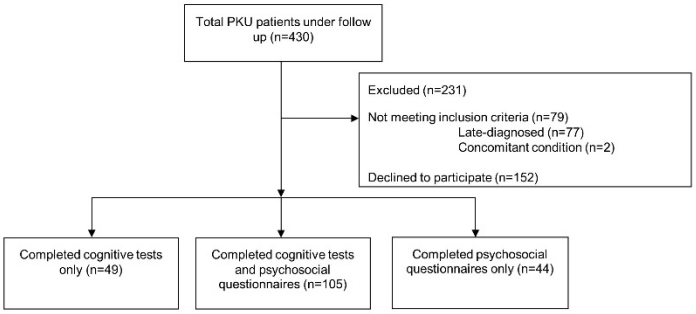


Figure 2

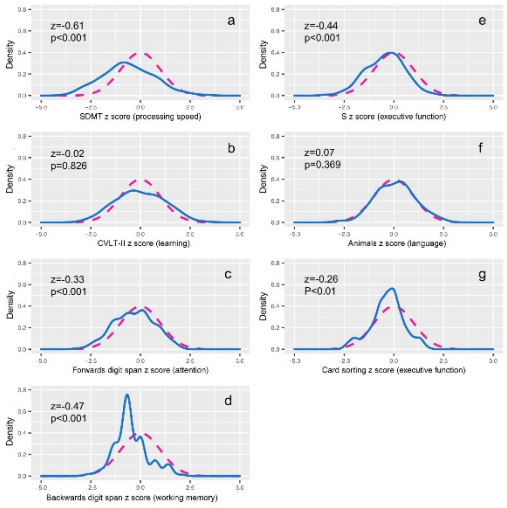
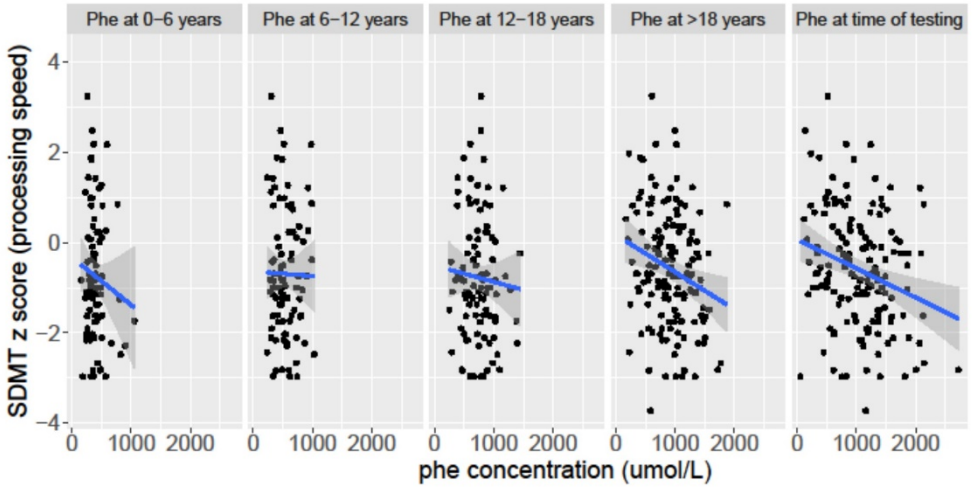


Figure 3

3-A. SDMT Scores (processing speed)



3-B. CVLT-II Scores (attention)