THE DIETARY MANAGEMENT
OF PHENYLKETONURIA

BY

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ABSTRACT

Dietary Management of Phenylketonuria

A wider understanding of the impact of each of the dietary components on blood phenylalanine concentrations in PKU may lead to improvements in management. Knowledge of the effects of such rigorous diet therapy on feeding behaviour is also important. In a series of studies, the effect of a number of dietary factors on plasma phenylalanine control and of diet on feeding behaviour was systematically investigated.

The key findings were: 1) there is wide variability in plasma phenylalanine concentrations which were not reflected in a single early morning phenylalanine measurement; 2) plasma phenylalanine concentrations were more influenced by the timing and dosage of protein substitute than by total energy or excess natural protein intake from 'freely' allowed foods; 3) repeated 4 hourly administration of protein substitute throughout 24-hours markedly reduced phenylalanine variability and led to lower phenylalanine concentrations; 4) 'free' use of fruits and vegetables containing phenylalanine between 51-100 mg/100g did not adversely affect plasma phenylalanine control; and 5) feeding problems were common, with almost 50% of young children with PKU exhibiting at least 3 feeding problems. These findings in PKU are important in the understanding of feeding behaviour; the interpretation **of** plasma phenylalanine concentrations; they increase and rationalise the range of 'free' foods; and will reduce 24-hour plasma phenylalanine variability, and thus, possibly increase dietary phenylalanine tolerance.

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ABBREVIATIONS

phe	Phenylalanine
EAR	Estimated average requirement
DRV	Dietary reference values
RNI	Reference nutrient intake
ANOVA	Analysis of variance
r	Pearson Product Moment Correlation Coefficient
SD	Standard deviation
SEM	Standard error of **the** mean
DH	Department **of** Health
MRC	Medical Research Council

Chapter Ⅰ

Introduction

Photograph not available in this web version.

Fig 1.1 Treated and untreated children with PKU.
The boy aged eleven is severely retarded while his 2 year old sister is normal.

1.1 Background information

Phenylketonuria (PKU) is an autosomal, recessive, genetic disorder (Costello *et al,* 1994). It is usually caused by a deficiency of the hepatic enzyme, phenylalanine hydroxylase (phenylalanine 4-mono-oxygenase, EC 1.14.16.1). This is a mixed function oxidase which catalyses the hydroxylation of phenylalanine to tyrosine, the rate limiting step in phenylalanine catabolism (Woo *et al,* 1986). Deficiency of this enzyme leads to an accumulation of phenylalanine, resulting in hyperphenylalaninaemia and abnormalities in the metabolism of many compounds derived from aromatic amino acids. The enzyme deficiency varies from complete absence of detectable activity, up to a residual activity up to 25% or more (Smith *et al,* 1991). Overall, the frequency among Caucasians is approximately 1 in 10,000 (Bickel *et al,* 1981) corresponding to a carrier frequency of about 1 in 50.

Untreated PKU leads to mental retardation (Fig **1.1),** hyperactive behaviour with autistic features, and seizures (Waisbren and Zaff, 1994). Treatment is a strict low phenylalanine diet, and if started within the first 3 weeks of life, irreversible mental retardation is prevented (Tyfield *et al,* 1990). Even when diet is started early, intellectual and neurological development may still be compromised both in early childhood and in adult life.

1.2 History

Classical PKU was first recognised by Asbjörn Fölling in 1934 (Fölling, 1934 a,b). He found and identified phenylpyruvic acid in the urine of two mentally retarded siblings. He later identified a further **8** children, from 430 mentally retarded patients, who had the same acid in their urine. Of the 10 detected children, there were **4** pair of siblings, suggesting this **was** a hereditary condition. Fölling suspected that phenylalanine might be the most probable source of the phenylpyruvic acid. He found that phenylalanine **and** a protein load each

separately increased phenylpyruvic acid excretion. He proposed that this was an autosomally transmitted recessive disorder of phenylalanine metabolism which he called *imbecillitas phenylpyrouvica.* In 1935, Penrose confirmed that this disorder was inherited as an autosomal recessive trait, and introduced the term phenylketonuria (Penrose, 1935).

It was a further 15 years before the biochemical basis of phenylketonuria was more specifically defined. In 1947, Jervis observed that the administration of phenylalanine to normal humans led to prompt elevation in serum tyrosine but this response was absent in patients with PKU (Jervis, 1947). In 1952, the enzyme system capable of converting phenylalanine to tyrosine was identified (Undenfriend and Cooper, 1952). A year later it was demonstrated that post-mortem liver samples from normal individuals were able to convert phenylalanine to tyrosine *in vitro,* while those from PKU patients were not (Jervis, 1953).

A low phenylalanine diet for the treatment of PKU was suggested by Woolf and Vulliamy (1951). This was first given to a 2 year old child with PKU at Birmingham Children's Hospital by Bickel, Gerrard and Hickmans (1953). The child had severe developmental problems; was unable to stand, walk, or talk; and spent her time groaning, crying and head banging. During treatment with a low phenylalanine diet there was a gradual improvement in the child's mental state. She learnt to walk, crawl, stand, climb on chairs, ceased head banging and her hair grew darker. A blind challenge with 5g L-phenylalanine led to the child becoming distressed and a recurrence of the head banging within 6 hours. Within 20 hours, she could no longer stand and could hardly crawl.

This case report was quickly followed by other studies demonstrating that a low- phenylalanine diet, if given early in infancy, prevented the mental and neurological deterioration otherwise almost inevitable in PKU (Woolf, Armstrong and Tyler, 1955; Blainey and Gulliford, 1956; Horner and Steamer, 1956; Low, Bosma and Armstrong, 1957;

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Woolf *et al,* 1958). However, there was an apparent inverse relationship between the time of starting therapy and clinical outcome. The later the diet was started; the less the benefit. These observations stimulated interest in neonatal screening programmes for phenylketonuria (Eisensmith and Woo, 1991).

The first screening programme, based on the urinary ferric chloride test, was implemented in California, **USA,** in 1957 (Centerwall, 1957), and later in the UK (Gibbs and Woolf, 1959; Boyd, 1961). Unfortunately the ferric chloride test had limited utility in widespread screening procedures, as the harmful elevation of blood phenylalanine precedes urinary excretion of phenylpyruvate (Armstrong and Binkley, 1956). False negative results were therefore common place. In 1963, Guthrie and Susie (Guthrie and Susie, 1963) discovered that blood phenylalanine could be easily measured in a semi-quantitative manner by a bacterial inhibition assay. This method required only a small amount of blood, and was specific, inexpensive and well-suited to analysing a large number of samples. It was adopted as a routine procedure to screen all newborn babies for PKU in most Western countries.

The original diet developed by Woolf and colleagues (Woolf and Vulliamy, 1951) has formed the basis of the current dietary approach. It consisted of a protein hydrolysate treated with activated charcoal to remove phenylalanine and tyrosine. The daily intake of casein hydrolysate was maintained relatively high because of possible inefficient protein utilisation. Extra tyrosine, methionine and cysteine were added together with vitamins and mineral salts (Table 1.1). It was supplemented with additional carbohydrate because it had already been shown that this enhanced utilisation of amino acids (Munro and Thomson, 1953). The protein substitute mixture was given in the form of a soup, and in older patients was only administered once daily. Cow's milk was given **as** the chief source of phenylalanine and a phenylalanine exchange system was introduced for older children who were allowed to replace cows milk with defined and strictly controlled amounts of cereal and potato.

A small range of foods were allowed freely. They included vegetables (except potatoes and pulses), oranges, apples, cornflour, sago, Kosher margarine, sugar, honey, jam, and low protein bread and biscuits made from gluten-free wheat starch. From 1957, the casein hydrolysate, supplementary amino acids, and mineral salts were provided by the National Health Service, but the gluten-free wheat starch had to be purchased by parents.

A UK conference on PKU in 1963 made a number of recommendations to the Medical Research Council about its management (MRC, 1963). Concern was expressed about over-treatment of PKU. There had been a number of reports of lethargy, listlessness, anorexia, poor weight gain, vomiting and even death which appeared to be due to phenylalanine deficiency in PKU infants and young children. For the first time guidance was given on desirable phenylalanine concentrations and frequency of blood phenylalanine monitoring. It was considered safer to maintain blood phenylalanine concentrations at between 1.5 and **4** mg/100 ml phenylalanine (90 and 240 μmol/l) which was slightly above the normal reference range (0.93 ± 0.27 mg/100 ml or 56 ± 16 μmol/l). One year later, the Medical Research Council and Department of Health and Social Security set up a National Register for PKU to document the clinical and intellectual progress of patients treated with a low phenylalanine diet. This was followed by the initiation of the United States Collaborative Study in 1967, which investigated the effectiveness of diet within the first 6 years of life and the need to continue diet beyond 6 years of age.

Over the last 30 years, the duration of diet has been the subject of much discussion and policy change. In the 1960's and 1970's, many PKU centres stopped diet as early 4 or **8** years, when it was argued brain development would be substantially complete. Initial reports on stopping diet were conflicting. Some studies showed no intellectual deterioration following discontinuation of diet therapy (Kang *et al,* 1970; Holtunan, Welcher, and Mellitts,

Table 1.1

Original 'milk' given to babies.with PKU (Woolf *et al*, 1958)

Ingredient	Daily Intake
Casein hydrolysate *	24g
L-Tyrosine	2g
DL-Tryptophan	1g
DL-Methionine	1g
Sucrose	90g
Cow's milk	0 – 200 ml
Double cream	85 ml
Calcium hydrogen phosphate	0.71g
Potassium chloride	0.65g
Sodium chloride	0.016g
Magnesium sulphate	0.165g
Sodium citrate	0.177g
Potassium iodide	0.00013g
Citric acid	0.08g
Water	to 850 ml
Other Supplements	
Choline chloride	100 mg
Inositol	216 mg
Vitamin B12	4 μg
Aneurine hydrochloride	0.5 mg
Riboflavin	0.5 mg
Pyridoxine	0.33 mg
Nicotinamide	3.33 mg
Ascorbic acid	40 mg
∝-Tocopherol	0.33 mg
Acetomenaphthone	0.5 mg
Biotin	0.17 mg
Folic acid	0.25 mg
Vitamin A	3000 iu
Vitamin D	500 iu
Ferrous sulphate	0.15g
Manganous sulphate	0.0008g
Zinc sulphate	0.0014g
Cupric sulphate	0.003g

* Acid hydrolysed casein passed through charcoal **to** remove phenylalanine and tyrosine.

1975; Chang *et al,* 1983; Saudubray *et al,* 1987); others showed falls in IQ (Smith *et al,* 1978; Hudson *et al,* 1970; Cabalska *et al,* 1973). The age of continuing either a strict or relaxed diet has gradually extended, and in the early 1990's diet for life was recommended in the UK (MRC, 1993a).

1.3 Classification of PKU

Phenylalanine hydroxylase deficient hyperphenylalaninaemia can be divided into two main types depending on residual enzyme activity.

1. *Classical ur severe PKU.* This is usually characterised by plasma phenylalanine concentrations over 1,000 μmol/l (an arbitrary threshold) at presentation (Scriver *et al,* 1995), but sometimes even in excess of 3,000 μmol/l. There is an almost a complete loss of enzyme activity (Ledley, Levy, and Woo, 1986) and in the liver phenylalanine hydroxylase activity is 0.3% or less of normal (Hilton *et al,* 1986). Historically, the condition is called 'classical' and in the UK almost 80% of all patients fall into this category of hyperphenylalaninaemia (Costello *et al,* 1994).

2. *Persistent hyperphenylalaninaemia.* This is a milder form of the disorder in that there is only a partial reduction in the activity of the phenylalanine hydroxylase enzyme from 2 to 5% of normal (Ledley *et al,* 1986). On a normal diet, the plasma phenylalanine will range from 120 to 1,000 μmol/l (Scriver *et al,* 1995). In the UK, 21% of all patients who have elevated phenylalanine have mild hyperphenylalaninaemia (Costello *et al,* 1994). Strict definition holds that there should be no urinary excretion of phenylpyruvic acid. However, in practice, there are 'borderline' patients who will occasionally excrete phenylpyruvic acid in easily detectable amounts, depending on dietary intake and other factors (Pollitt, 1994). Although this is sometimes referred to as benign hyperphenylalaninaemia, it has been shown to adversely affect intellectual ability

(Costello *et al,* 1994). It is recommended that all children who have phenylalanine concentrations of 400 μmol/l or above should follow a low phenylalanine diet (MRC, 1993b) at least in early childhood. **A** restriction of natural protein is not recommended for children with phenylalanine concentrations below this concentration.

There are also rarer forms of hyperphenylalaninaemia due to a deficiency of reductase or other enzymes involved in the biosynthesis of tetrahydrobiopterin (Kaufman *et al,* 1975, 1978; Leeming, Blair and Rey, 1976). These tetrahydrobiopterin deficiency disorders are sometimes referred to collectively as malignant `hyperphenylalaninaemias,` but the clinical picture is variable and some patients are only moderately affected, presenting late with mental retardation. **A** low phenylalanine diet is not effective. In Caucasian populations only 1-2% of cases of hyperphenylalaninaemias have tetrahydrobiopterin deficiency defects (Pollitt, 1994).

1.4 Does outcome in early-treated PKU relate to quality of control of blood phenylalanine?

Monitoring of PKU treated continuously and carefully following neonatal diagnosis has demonstrated that a low phenylalanine diet prevents mental retardation. Most early treated children who have started diet by 4 weeks of age fall within the broad normal range of general ability and attend mainstream schools (MRC, 1993a; Weglage *et al,* 1993). However, studies have indicated that the IQ of children with PKU is still significantly below population norms and that of unaffected siblings (Smith *et al,* 1990; Smith *et al,* 1991; Holtzman *et al,* 1986). Deficits have also been documented in information processing, abstract reasoning, high level problem solving, behaviour and linguistic ability. There is strong evidence to indicate that outcome is closely related to the quality of blood Phenylalanine control, particularly during pre-school and early school years (MRC, 1993a).

1.4.1 Effect on IQ

Three large national studies have investigated the quality of dietary control and effect on IQ in children.

1. United States Collaborative PKU study

This prospective longitudinal and randomised study was conducted in two parts at 19 treatment centres throughout the United States between 1967 to 1983. Part **A** compared strict with moderate restriction of phenylalanine during the first six years of life, while part B investigated diet continuation versus discontinuation after six years of age (Azen *et al,* 1996). *An* early report of 111 children at the age of four years, indicated that the children in the two treatment groups had a comparable mean IQ of 93. However, many of the children could not be maintained in the specified treatment category and there was considerable overlap of serum phenylalanine concentrations between the two groups (Dobson *et al,* 1977). In a follow-up report from the same study, the average IQ at 6 years of age for 132 subjects was 98, but was found to be significantly related to mother's IQ, the age at initiation of treatment and the phenylalanine concentrations during the first six years of life (Williamson *et al,* 1981).

In part B, a strong correlation between the age of loss of dietary control (ie. blood phenylalanine concentrations greater than 900 µmol/l) and cognitive test scores at 8 years and 10 years of age was demonstrated (Holtunan *et al,* 1986). It was also reported that the children with the lowest IQ's were those who had ceased dietary control before 6 years of age (Koch *et al,* 1987). Children who stayed on diet had higher reading and spelling scores at 10 years of age compared with those who stopped diet, but arithmetic, language and perceptual test scores declined uniformly in both groups from ages 6 to 10 years (Fishler *et al,* 1989). Overall, children with PKU, experienced more school problems (Koch *et al,* 1987), had lower IQ's, and lower Wide Range Achievement Test scores (WRAT) than their unaffected

siblings and parents (Holtzman *et al,* 1986). However, for children who were still on diet at the age of 8 years, the deficit was reduced for IQ when compared with their parents and did not exist when compared with their siblings. The authors strongly recommended the early initiation of dietary treatment and continuation throughout childhood (Azen *et al,* 1996).

2. German Collaborative PKU study.

One hundred and sixty five children were enrolled in the West German PKU collaborative study born between 1978-1984. They aimed to maintain plasma phenylalanine concentrations between 120 μmol/l and 240-360 μmol/l, but the means of median concentrations were greater than 360 μmol/l from from the age of 2 years onwards, and subsequently settled at concentrations of 420 μmol/l (Michel, Schmidt, and Batzler, 1990). Cluster analysis data was reported from this longitudinal study focusing on intelligence at **4,5** and 9 years of age. This was related to the quality of dietary control (ie. phenylalanine concentrations below 300 μmol/l, 360-600 μmol/l, and above 600 μmol/l) and in comparison with 200 healthy controls (Burgard *et al,* 1996). On average, all three levels of dietary control scored significantly lower than healthy age peers, but children with phenylalanine concentrations below 360 μmol/l had IQ's within the normal range. Full scale IQ's were not significantly different between good and moderate phenylalanine control but in the poor dietary control group, the mean IQ was 10 points below the scores of the healthy control group.

3. UK MRC PKU register

Smith *et al,* (1990) reported results from two cohorts of patients with phenylketonuria bom between 1964 and 1971 (cohort 1), and 1972 to 1980 (cohort 2). Fewer than 10% achieved good treatment results and the mean IQ of patients born in the mid 1970's was 8 points below the norm. Nineteen percent of patients in cohort 1 and 8 % of patients in cohort 2 had IQ's that were more than 2 standard deviations below the norm. The IQ fell by 0.28 of a standard

deviation (4 points) for each month delay in starting treatment, for each 300 μmol/l rise in phenylalanine concentration, and for each 5 months during the first 2 years which phenylalanine concentration was below 120 μmol/l. In a further report from the same two cohorts of patients, Smith *et al,* (1991) reported that the mean phenylalanine concentration rose from around 400 μmol/l during the first 4 years to above 900 μmol/l by 12 years. Those patients with good control throughout the first 8 years had better standard deviation scores (SDS) at 8 years, those with good control in the first 4 years but poor control between 4 and 8, did less well, and those with poor control throughout the first 8 years had the greatest IQ change. In those with good control after 4 years, IQ SDS stayed the same or showed a rise. In those patients born in the second cohort, an inverse association between intelligence and phenylalanine control persisted to at least 10 years, although the association was less marked than at earlier ages.

Adolescents and young adults

There is less clear evidence that high blood phenylalanine concentrations adversely affect IQ after the early teenage years. **A** further follow-up study from the UK MRC PKU register of the intellectual status of 192 patients at the age of 18 years, revealed that mean IQ's were significantly below population norms. Twenty seven percent had IQ's over two standard deviations below the estimated population mean. They showed a small decrease in IQ from 14 to 18 years of age. This was significantly related to average phenylalanine control between birth and 14 years and between 14 and 18 years. Only 5% of the patients had phenylalanine concentrations below 700 μmol/l. In this study there was no control group, and the authors suggest the apparent fall in IQ-SDS between 14 and 18 years was due to methodological problems. They concluded that the general ability in young adults is reduced in comparison with their peers, but is more closely related to phenylalanine control in early childhood than in later years (Beasley, Costello and Smith, 1994).

In Gemany, Schmidt *et al,* (*1996)* investigating a group of 51 young adults treated for PKU before **3** months of age, have reported that their mean IQ was 97. On average, their median phenylalanine concentration was below 300 μmol/l up to the age of 10 years, below 600 μmol/l up to 15 years and later approximately 900 μmol/l. Of the IQ's only 4% were more than **2** standard deviations below the norm, and IQ's did not deteriorate after the age of 10 years. The results were better than the UK MRC PKU follow-up study, and may reflect better German blood phenylalanine control in the first 10 years of life.

These studies would therefore indicate there is a close relationship between intellectual development in children with PKU and their dietary control. Intelligence decreases linearly with higher blood phenylalanine concentrations in children before mid-childhood. However, studies of early treated subjects with good dietary control suggest that the risk of intellectual deterioration declines dramatically in later childhood and adolescence.

1.4.2 Outcome measures other than IQ

Neuropsychological studies

Results of studies investigating problem solving, reaction times and sustained attention in **PKU** are summarised in Table 1.2 and 1.3. Deficits were found in information processing, abstract reasoning, both in conceptual and visuospacial areas and high level problem solving Waisbren *et al,* 1994; Brunner, Berch, and Berry, 1987; Pietz *et al,* 1992; Schmidt *et al, 1992).* Children with PKU who had remained on restricted diet performed better than those **who** had terminated diet, although most studies documented that performance was most closely related to concurrent phenylalanine concentrations or concentrations within the **previous** two years (Waisbren *et al,* 1994).

In **the** few neuropsychological studies in early treated adult patients (Ris *et al,* 1994; Pietz *et al,* **1993),** sustained attention has been shown to improve with lower blood phenylalanine

concentrations, but to deteriorate after dietary relaxation (Schmidt *et al,* 1994). Not all studies have reported abnormal neuropsychological test scores with high blood phenylalanine concentrations. Griffiths and co-workers (Griffiths *et al,* 1997) in a randomised crossover study, gave a high phenylalanine supplement for 3 months to sixteen 10 – 16 year old patients with PKU on diet. Mean phenylalanine concentrations were increased from 834 µmol/l to 1355 µmol/l. Intellectual ability, memory, and conduct were not affected by medium term hyperphenylalaninaemia.

Neurological symptoms

Neurological signs e.g. excessively brisk tendon jerks, ankle clonus and intention tremor have been noted in children with early treated PKU with high blood phenylalanine concentrations and in young adults returned to a normal diet (Smith *et al,* 1985; Thompson *et al,* 1990).

Severe neurological deterioration has been noted in at least 10 adults, all who had poor dietary control or had stopped diet in their early teens. Firstly, Villasana and co-workers (Villasana *et al,* 1989) reported neurological deterioration in 2 adult patients who stopped dietary treatment at *6* and 12 years respectively, although the latter did not start diet until he was 3 years of age. Neurological complications included rapidly progressive spasticity and increase in seizure frequency in the first patient and gradual deterioration in school performance, behaviour and concentration in the second. Thompson *et al,* (1990) described 7 adult patients in the UK, who developed quadriparesis, paraparesis, tremor and ataxia. Only 4 of these patients started diet early in infancy and all had stopped or relaxed diet before teenage years. **A** further case study, described a 19 year old male with PKU who developed a spastic paraparesis **8** months after stopping a low phenylalanine diet. He had always had poor dietary control, but also had a borderline low serum vitamin B12 concentration on presentation, although this was not attributed to his problem. His brother,

Table 1.2

Results of neuropsychological problem solving studies (adapted from Waisbren *et al*, 1994)

No. of patients with PKU	Authors	IQ mean (SD) / range	Intact functions	Problems	Blood phenylalanine control	Age: years mean (SD)/ range	Country
27	Brunner *et al*, 1983	96 (± 14)	Pure motor tasks	Concept formation, tactile-motor problem solving	Not given	9.5 (± 3.5)	USA
14	Seashore *et al*, 1985	90 (± 13)	Simple visual reaction time, speech	Higher cognitive skills and integrative skills	Diet stopped 5-6 years of age	9–14	USA
6	Pennington *et al*, 1985	91 (± 11)	Language, memory, attention	Conceptual skills and visuospatial skills	Mean phe 0–5y = 660 μmol/l. Diet stopped 5y	10.02 (9–14)	USA
11	Welsh *et al*, 1990	105 (±11)	Simple reasoning, visual memory	Higher level reasoning	Mean lifetime phe = 566 μmol/l. Concurrent phe = 565 μmol/l.	4.64	USA
62	Diamond *et al*, 1997	80–125	Spacial discrimination, recognition memory, visual attention	Complex memory, inhibitory tasks, executive function	360–600 μmol/l.	0.5–7	USA
11	Griffiths *et al*, 1998	105 (± 15) .	Normal executive function.		Mean lifetime phe = 341 μmol/l.	8.83 (5.1–11.9)	UK

Table 1.3

Results of reaction time and attention studies (adapted from Wainsbren *et al*, 1994)

No. of patients with PKU	Authors	IQ mean (SD) / range	Intact functions	Problems	Blood phenylalanine control	Age years mean(SD) / range	Country
10	Krause *et al*, 1985	67-114	Simple integrative functions	Reaction time improved with lower blood phenylalanine	High blood phe = >1,000 μmol/l Lower blood phe = <1,000 μmol/l	6–24	USA
22	Brunner *et al*, 1987	97	Not reported	Reaction time, encoding of information		16.4 (5.6)	USA
32	de Sonnerville *et al*, 1990	95 (± 17)	Simple addition	Sustained attention and complex addition correlated with higher blood phenylalanine	Patients either had blood phe > or < 570 μmol/l	8.7	Germany
28	Pietz *et al*, 1992	Not given	Not reported	Distinct MRI changes correlated with sustained attention deficits in 4 patients.	Not given	Adults	Germany
19	Schmidt *et al*, 1994	109	Not reported	Sustained attention and calculation abilities in both treatment groups	Patients either had blood phe > or < 630 μmol/l and 1320 μmol/l	8.7	Germany
16	Lou *et al*, 1992	105 (82-133)	Learning, visual-motor skills, sustained attention	Visual perception and visual short-term memory	Range blood phe 0y – 8y: 200–500 μmol/l 15y = 300–1,000 μmol/l	13.5 - 20	Denmark
20	Weglage *et al*, 1995	101.4 ± 10	Not reported	Difficulties in tasks which needed fine motor ability and concentration	Mean lifetime phe = 476 μmol/l Concurrent phe 583 μmol/l	10.9 (1.3)	Germany
20	Weglage *et al*, 1996	101.4 ± 10	Not reported	Selective and sustained attention	Mean lifetime phe = 476 μmol/l Concurrent phe = 583 μmol/l	10.9 (1.3)	Germany

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who also had PKU, had ceased dietary therapy, but his only neurological abnormality was a slight increase in his deep tendon reflexes of the lower limb (McCombe *et al,* 1992). He had poor dietary control reported through teenage years.

Neuroradiological studies (magnetic resonance imaging: (MRI)) have commonly revealed white matter abnormalities of the brain in older patients (Leuzzi *et al,* 1993; Bick *et al,* 1993; Thompson *et al,* 1993; Toft *et al,* 1994; Pietz *et al,* 1996). White matter abnormalities have been observed in both inpatients with late onset neurological symptoms and in symptom-free patients remaining on diet. Most of the MRI scans have revealed symmetrical patchy and/or band-like areas of enhanced signal intensity (Ullrich *et al,* 1994). These changes predominantly affect the posterior/periventicular white matter; areas which are thought to show the latest myelination in humans. More extensive lesions affecting the frontal and subcortical white matter have been reported (Ullrich *et al,* 1994). It has been shown that the extent of the MRI changes is closely and independently associated with the degree of hyperphenylalaninaemia at the time of investigation, and with the number of years since the subjects stopped taking a low phenylalanine protein substitute (Thompson *et al,* 1993).

Some workers have returned patients to a stricter low phenylalanine diet to investigate if the abnormalities in cerebral white matter are reversible (Cleary *et al,* 1995; Walter *et al,* 1997; Battistini *et al,* 1991). Cleary *et al,* (1995) documented significant improvement in MRI findings in the adult patients when blood phenylalanine concentrations were reduced below **900** µmol/l. The greatest changes were seen in those patients who maintained blood levels below 400 µmol/l. Complete reversal of moderate/severe brain MRI abnormalities were noted in **a** single patient with classical PKU who reduced blood phenylalanine concentrations to less than 450 µmol/l (Walter *et al,* 1997).

Although abnormal EEG finding have been noted in 12 – 15 year old PKU children, and advance with increasing age independantly of IQ development, there is no relation to either the age at onset or the quality of dietary treatment (Pietz *et al,* 1988).

Psychological outcomes

Behaviour problems have been documented in early treated 8 year old PKU children particularly in those with poor dietary control. Data from the UK/MRC study indicated that patients maintaining blood phenylalanine concentrations below 600 µmol/l from cohorts 1 and **2** (groups previously defined) were respectively 1.5 and 1.7 times more likely to have deviant behaviour whilst those patients maintaining blood phenylalanine concentrations over 600 µmol/l were respectively 2.5 and 1.9 times more likely to have behaviour problems (Smith *et al,* 1988). Patients with PKU were more likely to have mannerisms, show signs of anxiety, hyperactivity, be less responsive and more solitary than controls. Stevenson and co-workers (Stevenson *et al,* 1979) found that treated boys with PKU more frequently exhibited deviant behaviour than expected in the general population.

However, not all studies have demonstrated behaviour problems. Using a personality questionnaire, Weglage *et al,* (1994) demonstrated that the only difference between 10 year old children with PKU and a comparable control group was that they were more sensitive, empathetic and less masculine in their attitudes. Only six children from this study had poor dietary control (ie phenylalanine concentrations greater than 600 µmol/l after the age of **3** years).

Although there is evidence that early treated PKU young adults function in a productive manner in society (Koch *et al,* 1985; Schmidt *et al,* 1996), there are reports of psycho-social difficulties and psychiatric disorders in adults with PKU (Fisch *et al,* 1995; Ris *et al,* 1997; Waisbren *et al,* 1994). Waisbren *et al,* (1994) reported greater psychopathy in a group of 12

young women with PKU who had either started diet late or who had terminated diet for at least five years, when compared with an early treated group who had remained continuously on diet. In an early study, Waisbren and Levy (1991) described agoraphobia in five young adults with PKU. All five received a low phenylalanine diet only in early childhood, and three had started diet therapy after 3 months of age. However, when two of the women returned to a low phenylalanine diet, they had a dramatic reduction in symptoms.

Other studies have demonstrated no relationship between blood phenylalanine concentrations, duration of diet and psychological or psychiatric symptoms in early treated adults. Ris *et al,* (1997) studied psychosocial adjustment of 25 early treated patients with PKU aged 18 years and older. Most psychosocial measures were indistinguishable from sibling controls. However, on a self-report inventory of psychiatric symptoms, 20% of the patients demonstrated significant morbidity. Patients with PKU were more likely to be troubled by persistent impulses and thoughts that were unwanted as well as feelings of alienation and discomfort in interpersonal situations. There was no significant correlation with early dietary control.

In a controlled trial, in a group of 35 early treated patients with PKU, aged 17 to 33 years, Pietz *et al,* (1997) found psychiatric disorders in 25.7% of patients with PKU and in 16% of the control group. In the group with PKU, introversive symptoms were increased, whilst extroversive symptoms decreased. Problems included depressed moods, phobias, generalised anxiety, hypochondriac worries and anxiety at work. They were more common in women. No correlation was found between severity or pattern of psychiatric disorders and biochemical control. However, a restrictive controlling style of parenting was found to be a **risk** factor in the development of psychiatric symptoms.

Therefore, outcome in early treated subjects with PKU is not as good as first thought. Impairments are more closely linked with the quality of blood phenylalanine control than previously recognised. Studies have revealed intellectual, neurological and neuropsychological deficits as well as abnormalities of cerebral white matter in patients with early treated PKU. Whilst it is accepted that adverse effect of IQ can be reduced by implementation of diet as early as possible in the neonatal period, questions remain about the optimum blood concentration of phenylalanine in the treatment of PKU.

1.5 What should blood phenylalanine concentrations be in PKU?

Since the early 60's it has been PKU policy to maintain blood phenylalanine concentrations above normal (MRC, 1963) with upper limits of 600 μmol/l for young children. However, there is now compelling evidence that a persistently raised concentration of blood phenylalanine over 400 μmol/l in infancy and early childhood correlates quantitatively with a reduction in IQ (Smith *et al,* 1990; Smith *et al,* 1991). Costello *et al,* (1994) described the outcome at the age of 4 years of **24** untreated patients with 'atypical mild PKU' and blood phenylalanine concentrations consistently below 900 μmol/l. IQ was 9 points below population norms and IQ fell progressively by 6 points for each 100 μmol/l rise in phenylalanine concentration. More recently, Diamond *et al,* (1997) found in a 4 year longitudinal study, that children with PKU whose blood phenylalanine concentrations were three to five times normal (360-600 μmol/l) performed less well on tasks that required the working memory and inhibitory control abilities dependent on the prefrontal cortex.

However, although there is clear evidence that blood phenylalanine concentrations should not exceed 360-400 μmol/l in younger children (Table 1.4), aiming at 'normal' concentrations runs the risk of phenylalanine deficiency. There is evidence that this may be harmful to intellectual, neurological and nutritional status (Smith *et al,* 1990;

Table 1.4

Blood phenylalanine: normal and recommended concentrations in PKU

Age	Phenylalanine concentration μmol/l	Reference
Normal		
Term infants – breast fed 11 days	35–112	Scott & Wharton, 1994
Term infants – formula fed 11 days	37–94	Scott & Wharton, 1994
Infants and children	30–100	Green & Isherwood, 1994
Adolescents	mean 60 (SD ± 13)	Gregory *et al*, 1986
Adults	mean 58 (SD ± 14)	Scriver *et al*, 1985
PKU (recommended)		
Pre-school children: age 0-5 year	120–360	MRC, 1993b
School age children	120–480	MRC, 1993b
Children > 10 years	120–700	MRC, 1993b

Sutherland, Umbarger, and Berry, 1966; Sutherland, Umbarger, and Berry, 1970; Hanley, 1970). As a result of these findings, general UK recommended guidelines for desirable blood phenylalanine concentrations in young children are still above normal phenylalanine concentrations and are between $120 - 360 \, \mu mol/l$ (MRC, 1993b). **An** upper limit of $480 \, \mu mol/l$ is suggested for school age children, and $700 \, \mu mol/l$ in older children only because it is recognised that it is difficult to maintain lower blood phenylalanine concentrations in older patients. However, the recommended blood phenylalanine range of $120 - 360 \, \mu mol/l$ for younger children is still lower and narrower than has been maintained for over 30 years in PKU, and the newly recommended phenylalanine concentrations raise two issues:

      i)   Knowledge of the extent of diurnal variation of blood phenylalanine in PKU

      ii)   Appropriate blood sample timing in PKU

i) Knowledge of the extent of circadian variation of blood phenylalanine in PKU

Feigin, Klainer and Beisel (1967) first reported in normal infants a circadian periodicity of 'total serum amino acids' which was assumed to be more or less independent of meals. Information on blood phenylalanine fluctuations over 24 hours in PKU would be similarly important. As well as providing valuable information on the stability of blood phenylalanine concentrations in PKU, it would be helpful in the interpretation of single blood phenylalanine estimations.

Unfortunately, information on the circadian rhythm of plasma phenylalanine concentrations in patients with PKU is both limited **and** conflicting. Güttler *et al*, (1969) identified phenylalanine concentrations to be higher in the morning than evening in two children with PKU aged 3 years, but phenylalanine was measured at only two time points on each of three

consecutive days. Mean blood phenylalanine concentrations varied by almost 100 μmol/l/day between morning and evening, and evening serum phenylalanine was lower in the children with PKU than normal controls. In a single case study of a pregnant PKU patient, plasma phenylalanine concentrations were shown to vary by 200 μmol/l/day in 24 hours (Farquhar, Steven and Westwood, 1985).

In contrast, van Spronsen concluded that the daily fluctuation in plasma phenylalanine concentrations in PKU was small (van Spronsen *et al,* 1993). In this study, measurements were made at 30 minute intervals for 5 hours from before breakfast to 2 hours after the midday meal. In eight out of nine patients variation was less than 43 μmol/l, although one older patient did vary by 285 μmol/l. Phenylalanine concentrations increased rather than decreased over the period studied.

Results from non-PKU human studies (Feigin *et al,* 1967; Wurtman *et al,* 1968) and in an experimental animal (Feigin *et al,* 1969; Eriksson *et al,* 1989; Cai *et al,* 1994) are more consistent. In humans, the concentrations of most amino acids generally peak during the afternoon and reach their lowest point by 04.00h in the morning, whereas in rat this is reversed. Scriver and co-workers found the maximum concentration difference for phenylalanine to be less than *50%,* when phenylalanine concentrations were measured four times daily under fasting and non-fasting conditions in a group of 10 healthy adults (Scriver *et al,* 1985) **A** further study investigating plasma amino acid values in normal children showed little variation (Gregory *et al,* 1986). However, this study examined only inter-, subject variation and single blood specimens were taken at different times during the morning and early afternoon; dietary intake was uncontrolled.

ii) Blood sample timing in PKU

Even though the study results on phenylalanine variability are contradictory, it has been recommended that single blood phenylalanine specimens be taken at a standard time, ideally early morning when concentrations are likely to be at a peak (MRC, 1993b). It may be that single-point, early morning, phenylalanine concentrations are misleading in PKU, particularly if blood phenylalanine concentrations vary by as much as $100-200\,\mu mol/l/day$ as suggested by Güttler *et al,* (1969) and Farquhar *et al,(1985).* Theoretically if an early morning phenylalanine concentration is just above the MRC lower limit of $120\,\mu mol/l$, and decreases by over $100\mu mol/l/day$ during the day, phenylalanine concentrations could be very low for a considerable time in the second half of the day (MRC, 1993b). Similarly, if blood phenylalanine concentrations increase during the day, as suggested by van Spronsen's data (van Spronsen *et al,* 1993), it is possible that an early morning phenylalanine concentration just within the MRC upper limit in the early morning may exceed target levels during the remainder of the day (MRC, 1993b).

Further assessment of 24 hour variability of blood phenylalanine concentrations is therefore necessary both to test the validity of a single morning blood specimen and to determine if it is possible to maintain blood phenylalanine concentrations within the new guideline for recommended blood phenylalanine concentrations over a full 24 hour period.

1.6 Is the structure of the existing diet capable of consistently maintaining blood phenylalanine concentrations within target ranges?

The structure of the UK PKU diet is based on procedures developed in the 1950's and has subsequently developed by custom and practice. It has rarely been critically examined and no published study has attempted to evaluate existing diet therapy or modify practices systematically, even though many problems and anomalies have been recognised

(MacDonald, 1995; MRC, 1993a). The structure of the UK diet is quite different from systems used in other countries (Fig 1.2). It consists of four parts:

1. Daily allowance of dietary phenylalanine from measured quantities of moderate protein containing foods to provide phenylalanine requirements.
2. Provision of a phenylalanine-free protein substitute in order to meet nitrogen requirements.
3. Maintenance of a normal energy intake.
4. Provision of all vitamins and minerals to meet dietary requirements.

This system for allocating phenylalanine has been widely criticised, both within the UK (MRC, 1993a) and by dietitians in other countries (personal communication). It is thought more likely than systems in use in other countries to lead to higher or unstable phenylalanine concentrations. In the UK, children with classical PKU maintaining blood phenylalanine within the target range of 120–360 µmol/l, usually tolerate between 200-400 mg of phenylalanine daily, which would be found in 4–8g of natural protein. The system used does not permit high protein foods such as meat, fish, eggs, cheese, nuts, ordinary bread, biscuits and pasta, but allows a measured intake of moderate protein containing foods e.g. cereals, potato and some vegetables. These are given in the form of an exchange system, whereby one food can be exchanged or substituted for another of equivalent phenylalanine content. The idea of an exchange system was introduced as early as 1958 and the principles have changed little since. In the UK, a 50 mg phenylalanine exchange system is used. When phenylalanine data analysis of specific foods is not available, 1g protein is assumed to contain 50 mg phenylalanine (Barnes, 1994). **USA** and Australia use a 15 mg phenylalanine exchange system (Lyman and Lyman, 1960; Thompson, 1997), and calculate the phenylalanine content of all foods given in the diet. There are four specific problems which have been identified with the UK PKU diet:

Fig 1.2

Pictorial description of the low phenylalanine diet used in the treatment of PKU.
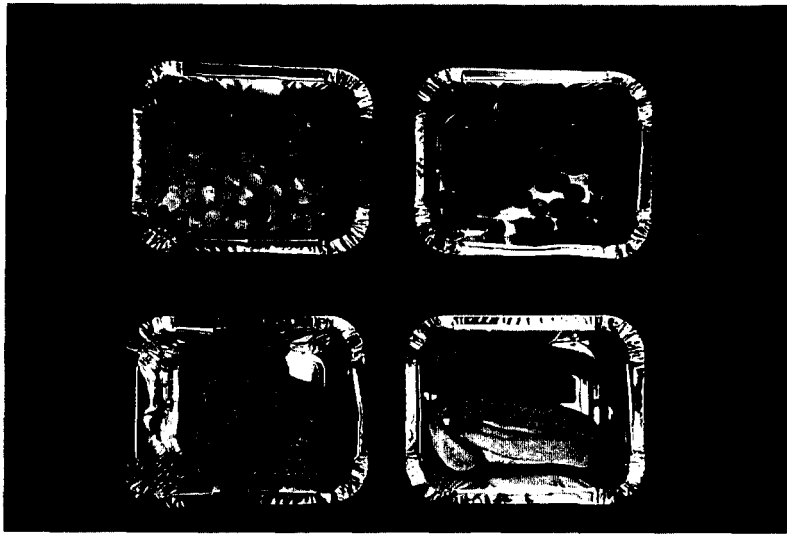
1: examples of 50 mg phenylalanine exchanges

2: examples of phenylalanine-free protein substitutes

3: examples of low phenylalanine foods allowed freely

1. **Phenylalanine per unit weight variability**

For many years, due to a lack of data on the phenylalanine content of different proteins, phenylalanine exchanges have been based on protein content rather than actual amino acid analysis. The assumption that 1g of protein contains 50 mg phenylalanine, does not apply to fruit and vegetables. Indeed most vegetables only contain 35 mg phenylalanine per gram of protein. However, there are a number of inconsistencies. For example, one gram of protein from asparagus contains 13 mg phenylalanine; whilst one gram of protein from spinach contains 140 mg of phenylalanine (Table 1*S).* Therefore, using exchanges adapted from protein data will provide only approximate intakes of phenylalanine. These may not be accurate enough to achieve and maintain the new blood phenylalanine lower target ranges.

The last published comprehensive phenylalanine analysis was in 1979 (Paul *et al,* 1980). It contained information about a limited range of foods. The most recent edition of 'The Composition of Foods' indicated that the protein value of many foods had slightly changed. In 1996, the Leatherhead Food Research Association and Laboratory of the Government Chemist analysed the phenylalanine content of 100 common fruit and vegetables (Weetch *et al,* 1997). Reassuringly, there was good correlation with the 1979 phenylalanine data. However, there remains wide gaps in knowledge about the phenylalanine content of many of the natural and manufactured foods commonly eaten by children and adults with PKU.

2. **Fruit or vegetable variability**

The category allocation of many fruits and vegetable in the PKU diet has caused particular controversy and debate. Some which contain less than 2.0g protein/100g are included as part

of the exchange system: others which contain more than 1.0g protein/100g are allowed as free foods. In particular, cauliflower and mushrooms which are relatively high in protein are allowed freely. **A** child who eats a portion of mushrooms and cauliflower within the same day, could be having the equivalent of two extra phenylalanine exchanges. There is no rational explanation for these practices. Indeed, in 1993, the MRC Working Group on PKU recommended that in order to maintain plasma phenylalanine concentrations within lower and narrower limits, these higher protein vegetables should be incorporated into the 50 mg phenylalanine exchange system in order to moderate their use in PKU. However, the contribution of protein or phenylalanine from these higher protein free vegetables to the PKU diet and the effect on control has not been assessed and warrants further investigation.

3. Free food definitions vary

The free food system accompanying the 50 mg phenylalanine exchange system has a number of limitations. There is no agreed definition of a 'free' food. The system generally includes all fruits and vegetables containing less than 50 mg phenylalanine/100g, other commodities which contain less than 0.3g protein/100g and low protein special products containing less than 20 mg/100g phenyalalanine. However, some higher phenylalanine/protein fruits and vegetables are permitted, and foods such as sauces (containing up to 2.0g/100g protein), butter (0.5g protein/100g), and some low protein breads and pasta (containing over 25 mg/100g phenylalanine) are allowed freely. The effect on plasma phenylalanine concentrations of such additional phenylalanine from free sources is unknown. Similarly, there are no data quantifying the contribution to the total diet of protein and phenylalanine from free foods, or it's effect on day to day plasma phenylalanine concentration variation.

Table 1.5

Phenylalanine content of fruit and vegetables

Quantity of vegetable and fruit providing 1g protein (Holland *et al*, 1991a)	Phenylalanine content per 1g protein (mg) (Paul *et al*, 1980)
30g boiled asparagus	13
100g canned tomatoes	19
145g raw cucumber	20
45g fried onions	22
25g baked potato weighed with skin	22
160g boiled turnip	22
45g boiled beetroot	29
170g boiled carrots	29
20g boiled broccoli tops	33
55g boiled potato	34
35g boiled brussel sprouts	35
35g boiled cauliflower	35
55g mashed potato	36
15g frozen peas	38
85g raw banana	40
35g roast potato	42
25g chips	43
100g boiled winter cabbage	51
35g canned kernels sweetcorn	53
20g baked beans	54
55g raw mushrooms	55
90g boiled runner beans	76
200g raw celery	84
45g boiled spinach	140

4. **Exchange system may be too sensitive**

Allocation of dietary phenylalanine by a single tier 50 mg exchange system may not be sufficiently sensitive to maintain phenylalanine concentrations within the narrow limits recommended by the MRC (MRC, 1993b). The use of alternative exchange systems need consideration. Possible options include: a) a two tier exchange system using a combination of 50 mg and 25 mg exchanges; and b) calculating the phenylalanine content of all foods given to a child with PKU. Both options would ensure that most of the existing 'free' vegetables which contain significant quantities of protein would be included as part of a measured phenylalanine exchange system. Although there is merit in these systems, they would make the diet even more complicated and restrictive. They may potentially increase parental anxiety, cause feeding problems in younger children, and lead to cheating in older children as a result of the fewer free foods from which to choose. **A** two-tier phenylalanine exchange system was used in the UK previously, but abandoned in the early 1980's. No adverse effect on control was reported, although the effect of this dietary change was not specifically monitored. There has been no attempt to compare blood phenylalanine control and the psychosocial outcome **of** different dietary regimens for allocating phenylalanine.

1.7 Other dietary factors which may affect control in PKU

1.7.1 Protein substitutes

It has been suggested that the composition, quantity and diurnal distribution of the amino acid intake could be important in PKU and influence blood phenylalanine control (MRC, 1993a). Protein substitute was found to suppress blood phenylalanine concentrations as early as 1961 (O'Daly, 1961). Over 75% of the amino acid requirements (except phenylalanine) is provided in the form of a protein substitute. They also provide all tyrosine requirements.

presentation and composition of protein substitutes.

They can be categorised into six groups:

i   Protein hydrolysate powders – rarely used in the UK.

ii   Amino acid powders: ± carbohydrate, and without vitamins and minerals.

iii   Amino acid powders: with added vitamins and minerals but minimal carbohydrate.

iv   Amino acid powders: with added carbohydrate, ± fat, vitamins and minerals

v   Amino acid capsules and tablets: without added carbohydrate, vitamins and minerals

vi   Amino acid bars: without vitamins and minerals.

Their presentation and composition may affect phenylalanine control in 3 ways. Use of protein hydrolysate or L-amino acids as primary sources of protein equivalent may be inefficiently utilised and inadvertently affect control (Acosta, **1996);** protein substitutes with added carbohydrate may reduce leucine oxidation and increase net protein synthesis (Motil *et al* **1981);** and poor acceptability of taste or presentation may affect compliance and therefore phenylalanine control (Clark *et al,* **1994).** The main types and features of protein substitute available in the UK are identified in Table **1.6.**

Requirements

Insufficient protein substitute may affect phenylalanine control and growth. There is little available data to help assemble rational recommendations and therefore, there is much debate regarding requirements. In the UK, a high protein substitute intake is favoured and it is recommended that children under 2 years should take at least 3g/kg body weight/day; those over 2 years are recommended to take 2g/kg body weight/day. This is quite different to many centres in the USA and other European countries (Prince, McMurry, Buist, 1997; Schaeffer *et al,* **1994;** Przyrembel **1996).**

Table 1.6

Protein substitutes: presentation and composition

Type	Amino acid g/100g	Protein g/100g	Energy kcals/ 100g	kJ/ 100g	Added CHO g/100g	Added fat g/100g	Added vits/ mins	Advantages	Disadvantages	Presentation	Supporting research studies
L-Amino Acids PK Aid 4 (SHS) Aminogran Food Supplement (UCB)	93.2 97.2	77.8 -	326 400	1387 1675	Nil Nil	Nil Nil	Nil Nil	• Flexible • Low bulk • Easy to prepare	• No carbohydrate • Poor palatability • Compliance with separate vits/ mineral supplement poor	Powder (400g tub) Powder (500g tub)	Associated with selenium deficiency (Lipson *et al*, 1988, Wilke *et al*, 1992)
Phlexy 10 Drink Mix (SHS)	50	41.7	343	1456	44	Nil	Nil	• Flavoured • Palatable • Convenient • Moderate bulk • Flexible	• No added vits/ minerals. • Available in only 1 flavour	20g sachets (powder)	Shown to result in better compliance and improved phe control when compared with other protein substitutes (Clark *et al*, 1994)

CHO= carbohydrate
phe = phenylalanine
vits = vitamins

Table 1.6 (continued)

Type	Amino acid g/100g	Protein g/100g	Energy kcals/ 100g	Energy kJ 100g	Added CHO g/100g	Added fat g/100g	Added vits/ Minerals	Advantages	Disadvantages	Presentation	Supporting research studies
L-Amino Acids with added vits/minerals but minimal carbohydrate											
Milupa PKU2 (Milupa)	80.1	66.8	300	1275	8.2	Nil	Some	• Low bulk • Age specific • Easy to prepare	• Inflexible • Does not contain all vits/mins. • Minimal carbohydrate • Poor palatability • Poor compliance • Unsuitable for all age groups	Powder (500g)	
Milupa PKU 3 (Milupa)	81.6	68	288	1222	3.9	Nil	Some			(Powder (500g)	
L-Amino Acids with added CHO/vits/minerals											
XP Maxamaid (SHS)	30	25	300	1260	51	Nil	Yes	• Age specific • Easy to prepare • Contains all added vits/ minerals	• Inflexible • Bulky • Poor palatability • Poor compliance • Unsuitable for all age groups	Powder (500g)	Growth normal (Acosta et al, 1996, Wardley et al, 1988) XP Maxamaid associated with low ferritin concentrations (Bodley et al, 1993)
XP Maxamum (SHS)	47	39	290	1226	34	Nil	Yes			Powder (500g)	

33

Table 1.6 (continued)

Type	Amino acid g/100g	Protein g/100g	Energy kcals/100g	Energy kJ/100g	Added CHO g/100g	Added fat g/100g	Added vits/mins	Advantages	Disadvantages	Presentation	Supporting research studies
Novel Presentations											
Phlexy 10 bar (SHS)	23.8	19.8	371	1562	48.8	10.7	Nil	• Palatable • Convenient	• Bulky • No vits/minerals • High calorie • Product fatigue	Bar (42g)	Bars been shown to score highly on acceptability rating but did not improve compliance (Prince *et al*, 1997)
Phlexy 10 Capsules (SHS)	50	16.7	176	747	2.3	Nil	Nil	• Convenient • No mixing	• Need large numbers • No vits/ minerals • Suitable for older patients only	Capsules (0.5g amino acid per capsule)	In a single case, compliance better than with conventional supplement (Keckskemethy *et al*, 1993). Two out of 19 patients preferred capsules to conventional products (Clark *et al*, 1994)

There is evidence that L-amino acids may be poorly utilised. Following ingestion of L-amino acids, with and without whole protein, there are higher and more rapid rises followed by more rapid declines in individual plasma amino acid concentrations than with whole protein alone (Gropper *et al,* 1993). This must inevitably lead to greater loss of amino acids both by destruction in the liver and excretion in the urine (Woolf, 1962). Indeed, it has been reported that nitrogen losses were significantly greater in subjects receiving L-amino acids as their source of protein equivalent than in subjects receiving the same amount of nitrogen from whole protein (Jones *et al,* 1983).

In two independent PKU studies, Acosta and Yannicelli, (1994) and Kindt *et al,* (1984) have shown that phenylalanine tolerance increases when total protein intake from the protein substitute increases. Acosta *et al,* (1994) demonstrated in 25 infants with PKU that when an infant protein substitute containing 3,12g of protein per *100*kcals was administered, the total daily phenylalanine intake increased and blood phenylalanine concentrations were lower than when a lower protein substitute was used. Kindt *et al,* (1984) fed two groups of children with PKU different quantities of total protein. One group adhered to the US Recommended Dietary Amounts (**RDA,** 1980) and the other group to the lower safe levels recommended by the Joint FAO/WHO Expert Committee (1973). The RDA group tolerated 3–5 mg/kg body weight /day additional phenylalanine. Also, it was found that two children given the FAO safe level of protein had retarded growth velocity, and high fasting glycine and alanine amino acid values. This did not occur in the group of children fed the US **RDA** for protein.

Furthermore, recent studies from Europe have reported impaired growth in infants and young children with PKU (Dhont *et al,* 1995; Schoeffer *et al,* 1994; Verkerk *et al,* 1994; Weglage *et al,* 1994) which could have been related to a low protein substitute intake. In the USA, Prince *et al,* (1997) found that in a group of patients aged 4–10 years the actual amino acid intake decreased from only 0.9 g/kg body weight/day to as little as 0.4 *g/kg* body

weight/day in children over 4.2 years of age. Growth and nutritional biochemistry were not adversely affected although mean serum phenylalanine increased from 380 to 480 μmol/l. The authors suggested that the increase in serum phenylalanine was just a reflection of increasing patient age, but equally it could have related to reduced intake of protein substitute.

Therefore, high quantities of protein substitute may lead to increased nitrogen retention (Kindt *et al,* 1984), improved phenylalanine tolerance (Acosta *et al,* 1994; Kindt *et al,* 1984), and help prevent an imbalance of amino acid transport across the blood brain barrier (Pratt, 1980).

One study does not support a high intake of protein substitute. Clemens *et al,* (1991) gave ten older patients aged from 15 to 37 years three different levels of protein from a standard phenylalanine free amino-acid mixture for three months each. This consisted of a) 1.5 g/kg of protein from a essential amino acids and tyrosine only; b) 0.75 g/kg from essential amino acids and tyrosine; and c) 0.65 g/kg of protein from essential amino acids and tyrosine. For all three levels of protein intake, there was no sign of amino acid imbalance or catabolism. On the basis of these results, Clemens suggested that providing a protein source of high biological value similar to the essential amino acid mix used in the study is given, the overall quantity of the protein substitute may be reduced (Clemens *et al,* 1991). However, all these patients were on more natural protein than younger children with PKU and intake varied between 9–26 g/day. Higher natural protein intakes should be taken into account when calculating protein substitute requirements.

Timing of protein substitute intake

The effect of timing of protein substitute intake on blood phenylalanine concentrations has not been systematically studied. It has been recommended that protein substitute should be

taken three times daily with main meals (Barnes, 1994). This is a demanding routine particularly in school-age children and adolescents. There is however, evidence that infrequent administration of large doses of protein substitute increases nitrogen excretion as well as oxidative utilisation of amino acids. In nine patients with PKU and one with hyperphenylalaninaemia, protein substitute was taken in one or two doses on day one, and on day two in three equal doses with meals (Schoeffer *et al,* 1994). In eight patients, excretion of nitrogen decreased from between 6.3-12.4g/day to 4.7-10.8g/day when the total amount of amino acid mixture was given in smaller and frerquent doses. Herrman *et al,* (1994) demonstrated in a single subject with PKU, using the ^{13}C-leucine breath test to measure oxidative utilisation of ^{13}C-leucine, a 33% dose of protein substitute produced ^{13}C-leucine elimination rate of 9.5% compared with 19.6% when protein substitute was given as a single larger dose. Evenly spread distribution of protein substitute intake may lead to better blood phenylalanine control, although there is no evidence to support this.

1.7.2 Energy

The importance of dietary energy in maintaining good blood phenylalanine control has not been demonstrated, though the influence of energy on protein utilisation and nitrogen balance is well documented (Millward and Rivers, 1987). In response to very low energy intakes net protein deposition is eliminated, and all dietary amino acids are oxidised. It is generally believed that the stimulation of tissue protein deposition occurs in response to intakes of dietary energy as well as protein, with dietary carbohydrate more effective than fat (Munro, 1964). Garza, Scrimshaw and Young (1977) fed six subjects a diet providing the safe level of protein intake (0.57g/kg body weight) and showed they were unable to maintain nitrogen balance when their diet provided their calculated energy requirement. In fact, they were only able to maintain nitrogen balance when their energy intake was increased to such an extent they showed a persistent weight gain. It may be possible that an increase in energy intake in PKU may enhance net protein synthesis. However, the effect of this has not been

specifically studied in PKU, and there are no data which document the energy intake of PKU

patients on the UK diet or have measured its effect on blood phenylalanine control.

1.8 What do PKU children actually eat?

There are potentially many other dietary factors which may affect blood phenylalanine

control, However, it is difficult to assess the effect of any of these as there are no published

data documenting macronutrient intake and timing of protein substitute intake in children

with PKU in the UK. It is inappropriate to suggest dietary modifications until actual intake

of children with PKU and effects on blood phenylalanine control are assessed. Moreover,

there are few data describing dietary habits, food patterns, food preferences, utilisation of

low protein special products and administration of protein substitute in PKU. It is important

to understand fully the application and practices of diet therapy so that further advances can

focus on what is tolerable, acceptable and practical for the patient.

1.9 What is the effect of the PKU diet on normal eating behaviours?

The low phenylalanine diet is perhaps one of the most difficult diet therapies designed for

patients and parents. It is based on few normal foods, a limited number of low protein

specialised products and an unpalatable protein substitute. Admittedly, children with PKU

start this diet therapy shortly after birth, so should not develop a taste for normal foods, but

their food looks and tastes very different from that for the rest of their family and friends.

A PKU diet can be made appetising and acceptable, but this requires cooking skills, time,

imagination, and a wide range of cooking appliances. There are no ready-made meals which

can be purchased from supermarkets, so all meals have to be prepared from basic food

ingredients. Some of the special low protein ingredients, such as the flour mixes, are very

difficult to work with, as their basic structure as been altered by removing protein. **As** a

consequence, low protein cooking failure rate appears high, which in turn leads to

disillusionment and the abandoning of low protein special cooking, except for the most dedicated of parents. Parents may then keep meals very simple and it is not uncommon for young children to be offered potatoes and vegetables only. Boredom, hunger and poor compliance may be the consequence.

Food and mealtimes are an important feature of everyday life. Most of the food eaten by the rest of the family is unsuitable for a child with PKU and there may be a tendency to feed the child separately, so the family communication and rapport of mealtime is lost. In addition, the process of food shopping and preparation may not be shared with the child, which may lead to disinterest in their diet. Even cooking at school is based on normal food so there is little opportunity to learn about PKU cooking and diet. It is almost impossible to go for a meal in a cafe, restaurant or party and find food which is suitable. This means that children with PKU either have to take their own food, refrain from eating or even eat small amounts of forbidden food.

It is, therefore, probable that such a restrictive diet therapy may adversely affect normal feeding behaviour particularly in young children. Surprisingly, no studies have investigated the effects of diet on feeding behaviour. There is a possibility that an over-restrictive diet and routine may adversely affect blood phenylalanine control either through lack of adequate calories or a rebellious child resorting to cheating. Therefore, it is important to measure the effect of this enforced, abnormal, diet on eating behaviour.

1.10 Summary

In PKU, intellectual and neurological outcomes are not as good as were first thought. These impairments are more closely linked with the quality of plasma phenylalanine control than previously recognised. This had led to recommendations that blood phenylalanine concentrations are kept lower and within narrower target ranges. There is some suggestion

that blood phenylalanine concentrations may undergo a wide diurnal variation, and it may be difficult to maintain blood phenylalanine concentrations which remain within target ranges. However, the evidence is limited and contradictory and circadian variation poorly understood. In addition, there is concern that the structure of the existing UK PKU diet may be incapable of achieving low, stable blood phenylalanine concentrations. Moreover, the PKU diet is complex and many factors may affect blood phenylalanine control, including allocation of dietary phenylalanine, contribution of phenylalanine from free foods, energy intake and timing and dosage of protein substitute intake. The effect of each of these factors on blood phenylalanine control has not been systematically studied. It is important that their impact on phenylalanine concentrations is documented before the diet can be modified and improved. Finally, the PKU diet is rigid, inflexible and can be unpalatable. If the diet is to be reconstructed or revised, it is essential that its effect on eating habits and feeding behaviour is established, to ensure it is tolerable and acceptable.

1.11 Summary of questions addressed in PhD thesis

1. Which dietary factors affect variation in plasma phenylalanine control in PKU?

An observational, longitudinal study was conducted in well-controlled subjects with PKU to study the effect of three factors thought to influence plasma phenylalanine concentrations in PKU; total energy intake; protein intake from natural foods allowed freely in addition to allocated phenylalanine exchanges; and the distribution of protein substitute throughout the day.

2. What is the effect of fruits and vegetables containing 1 – 100 mg phenylalanine on plasma phenylalanine control?

This was studied in two parts:

1) **A** prospective, intervention, cross-over trial investigating the effect of protein versus phenylalanine analysis for dietary potato exchanges on blood phenylalanine control in PKU.

2) **A** prospective, intervention, cross-over trial investigating the free use of fruits and vegetables containing 1-100mg/100g of phenylalanine on blood phenylalanine control.

3. What is the variation of blood phenylalanine concentration in PKU over 24 hours and is a single blood phenylalanine concentration representative of a 24 hour period?

An observational study was conducted investigating the 24 hour variability of plasma phenylalanine concentration in well-controlled in children with PKU.

4. What is the effect of even distribution of protein substitute on blood phenylalanine concentrations throughout the day and night?

It was hypothesised that administration of protein substitute over longer hours and in more frequent doses would lead to improved phenylalanine control.

This was examined in a two part study:

i) Part 1. **A** randomised, crossover study comparing three different protocols of protein substitute administration during the day in well-controlled subjects with PKU.

ii) Part **2. A** randomised, crossover study comparing three different protocols of protein substitute administration during both the day and night in well-controlled subjects with PKU.

5. What is the effect of the PKU diet on normal eating behaviour?

An observational, controlled study investigating the feeding behaviour of a group of unselected PKU children was conducted in a group of 15 PKU subjects aged 1-5 years old.

Chapter 2

Factors affecting the variation in plasma phenylalanine in patients with phenylketonuria on diet

2.1 Introduction

Many factors are thought to affect the day-to-day variation in plasma phenylalanine concentrations in PKU. They include additional or 'hidden' phenylalanine from 'free' foods, dietary energy, and timing of protein substitute intake. However, their impact on plasma phenylalanine concentrations has not been systematically studied, nor has the daily variability of each of these individual dietary components been investigated.

The aims of this study were to assess the effects of plasma phenylalanine control of variations in phenylalanine intake from 'free foods', as well as the effect of total energy intake and the timing of protein substitute administration. In a longitudinal, observational study, the effects on dietary intakes on plasma phenylalanine concentrations were compared over six months in a group of well-controlled children with PKU.

2.2 Methods

2.2.1 Subjects

Nineteen children with classical PKU were recruited. Eighteen were Caucasian and one Afio-Caribbean. There were three inclusion criteria: maintenance of plasma phenylalanine concentrations within the ranges recommended for age group by the MRC Working Group for 70% of the six month before entering the study; age 1 to **16** years; and parental ability to take skin puncture blood specimens at home by thumb prick. There were fifteen girls and four boys, with a median age of **6.6** years (range 1 to **16** years). All the children were on a strict low phenylalanine diet comprising: (1) a dietary phenylalanine allocation using a 50 mg phenylalanine exchange system (50 mg $\cong$ 1g protein); (2) a phenylalanine-free protein

substitute; and (3) low phenylalanine foods e.g. most fruits many vegetables and special low protein products, or so called "free" foods permitted in usual quantities.

The median number of 50 mg phenylalanine exchanges allocated was five per day (range 3 to 17), or 5g natural protein (Table 2.1). The following amounts of total protein/kg/body weight from protein substitute and phenylalanine exchanges were allocated: children 1 year of age, 3.0g/kg; 2–5 years, 2.5g/kg; 6–10 years, 2.0g/kg; over 11 years of age, 1.5g/kg. The brand of protein substitute used was chosen by the patient and was administered either as a paste or drink. Twelve patients used *XP* Maxamaid (Scientific Hospital Supplies), three patients used Aminogran Food Supplement (UCB Pharma), three used Phlexy 10 Drink Mix (Scientific Hospital Supplies), and one used XP Maxamum (Scientific Hospital Supplies).

The study was approved by the Committee of Medical Ethics of South Birmingham Health Authority. Informed consent was obtained from all parents and from the patients (where maturity and understanding were appropriate).

2.2.2 Methods

Assessment of phenylalanine and energy intakes

In total, parents performed serial 21-day weighed food intakes over 6 months at home. In months 1,3, and *5,* parents weighed and recorded their child's food intake over **3** days from Monday to Wednesday inclusive. In months 2,4, and **6,** weighed food intakes were performed over 4 days from Thursday to Sunday inclusive. Parents recorded only food actually eaten, and any waste food was reweighed and deducted from the total weight. They also described cooking techniques, precise food brand, kept nutritional labels of

Table 2.1

Age and allocated 50 mg phenylalanine exchanges of patients

Patient No.	Age (years)	Sex (male/female)	Number per day of 50 mg phenylalanine exchanges	Recommended phenylalanine concentrations (µmol/l) (MRC, 1993b)
1	3	F	5	120–360
2	3	F	3	120–360
3	12	M	8	120–700
4	16	F	14	120–700
5	7	F	5.5	120–480
6	3	F	5	120–360
7	1	F	7	120–360
8	4	F	4	120–360
9	2	F	3.5	120–360
10	4	F	3	120–360
11	1	M	3	120–360
12	14	F	12	120–700
13	9	F	3	120–480
14	9	F	12.5	120-480
15	16	F	17	120–700
16	6	M	4	120–480
17	9	F	9	120–480
18	3	M	3.5	120–360
19	3	F	3	120–360

manufactured foods eaten, and identified individual ingredients of any home made dishes prepared. Parents were instructed to use Salter electronic scales. Immediately after each study period, parents were visited at home to check food records. Each meal and snack consumed by the child was reviewed with the parents to check accuracy. In addition, the weighing technique of each of the mothers was assessed and confirmed as adequate by weighing 10 different food items at the start of the study. Timing and quantity of protein substitute consumed were also recorded prospectively.

Nutritional analysis of food intake was calculated using the Microdiet computer programme based on *McCance & Widdowson's* *"The Composition of Foods"* (Holland *et al,* 1991), with supplementary analysis data provided by the Royal Society of Chemistry (Holland *et al,* 1991a; Holland, Unwin, and Buss, 1991b; Holland Unwin, and Buss, 1989; Holland, Unwin, and Buss, 1988). Additional information on protein substitutes and low protein special products was provided by manufacturers and added to the database. All food coding was done manually and entered into the computer by the same researcher. To minimise error, such as wrong amounts, incorrect codes, amounts or codes being entered twice, omitted data, transcription errors, all data codes were checked twice and a *summary* sheet of all food items for each day's food diary was confirmed after the data had been entered into the computer. Excess natural protein intake was calculated in addition to the prescribed 1.0g protein (50 mg phenylalanine) exchanges. Energy intake was calculated as a percentage of the estimated average requirement for energy (EAR) (DH, 1991) and total protein intake as a percentage of reference nutrient intake (RNI).

The phenylalanine analysis was calculated from data from the *First supplement to McCance & Widdowson*s *·· The Composition of Foods ··* (Paul *et al,* 1980) and the Leatherhead Food Research Association and Laboratory of the Government Chemist (Weetch *et al,* 1997) fruit and vegetable phenylalanine analysis. When specific phenylalanine analysis data were not available it was assumed that 1g protein yielded *50* mg phenylalanine (Barnes, 1994).

Assessment of plasma phenylalanine concentrations

Twice daily serial skin puncture (heel, finger or thumb) blood specimens for phenylalanine were taken at home on the same days as the weighed food intake. Blood specimens (300 µl) were taken at the same time each day, standardised for each patient, but always pre-breakfast **and** pre-evening meal. The blood specimens were collected in Starstedt CB300 heparinised tubes by parents and immediately posted to the hospital laboratory to arrive within 24 hours of collection. Blood collection technique had been taught and competence assessed by a specialist nurse. All patients were free from intercurrent infections on the days of the study. If a patient became ill immediately before or during the study days, the plasma phenylalanine measurements and weighed food intake were deferred until the patient had recovered.

Blood specimens were analysed for phenylalanine by high pressure liquid chromatography (HPLC) (Atherton and Green, 1988) in the Birmingham Children's Hospital screening laboratory. Specimens were centrifuged on receipt, and the plasma was removed and stored frozen (-20°C) if it was not analysed without delay.

The plasma was precipitated in 50 µl--aliquots of equal volumes of plasma and perchloric acid containing an internal standard of alpha-methyl phenylalanine. The supematant was

frozen to enhance protein precipitation and then centrifuged. The supernatant was then injected using an auto-sampler onto the HPLC with an aqueous calibration curve. Internal quality control specimens were processed identically. The phenylalanine and internal standard were detected by their absorption of ultra-violet light at a wavelength of 214 nm. The phenylalanine concentrations were calculated by the comparison of its absorption with those of calibrating standards. The assay quality was checked by the National External Quality Assurance Scheme.

2.3 Statistical analyses

Statistical analysis was by Pearson Product Moment Correlation Coefficient (r) when comparing intake of dietary protein from 'freely' allowed foods, timing of protein substitute, and energy intake on plasma phenylalanine concentrations. Paired *t* tests were used to compare pre-breakfast and pre-evening plasma phenylalanine concentrations and natural phenylalanine and protein intake. Multiple regression was used to compare the effect of intake of dietary protein from 'freely' allowed foods, timing of protein substitute and energy intake on change in plasma phenylalanine concentrations between pre-breakfast and pre-evening meal.

2.4 Results

2.4.1 Dietary intake

protein intake

Natural protein intake

Assuming that 1.0g protein is equivalent to 50 mg of phenylalanine, the allocated median daily natural protein intake was 5g (range 3-17g), although the actual was 7.8g (range 4.1-21.5g) daily. **A** comparison of prescribed protein intake with actual intake (derived from weighed food results) showed that patients consumed a mean (SD; range) of 49% (37%, 12–162%) extra natural protein from phenylalanine exchanges to the prescribed amount. Only a mean of 6% (SD 9.5) of the excess protein was from miscalculation of phenylalanine exchanges by the parents. There was a very large day-to-day variation for some patients (Fig 2.1) with a mean (SD; range) coefficient of variation of 60% (32%; 11–150%) for the entire group of patients. There was no correlation with age. The median natural protein intake was distributed as follows: 27% breakfast and pre-midday; 27% lunch and pre-evening meal; and 45% pre-evening meal and bedtime. The five most common sources of natural protein were crisps, breakfast cereal, potatoes, chips and tinned spaghetti.

Actual phenylalanine intake

Using phenylalanine analysis data, and when unavailable, extrapolating phenylalanine analysis from protein analysis, the actual phenylalanine intake was a median of 330 mg daily (range 170–1,000 mg). This equates to 6.6g (range 3.4-20g) natural protein daily and was significantly less than actual protein intake ($p < 0.0001$), but still more than allocated

phenylalanine exchanges by a mean of 31% (SD 32, range −9 to 113%). However, there was close correlation between actual protein and phenylalanine intake (r = 0.975; p <0.0001) (Fig 2.2). The mean (SD; range) coefficient of variation of phenylalanine intake was only 20% (6%; 8 to 31%) which was less variable and lower when compared to dietary protein (p <0.0001) (Fig 2.3). Although phenylalanine per kg/bodyweight decreased with age, the correlation was not significant (p = 0.0668).

Protein intake from protein substitute

The median protein intake from protein substitute was 36g (range 13−668) daily. The median percentage of protein from protein substitute was 84% (range 49−87%). Only two patients reported that they did not consistently complete the protein substitute.

Total protein intake (from natural protein and protein substitute)

The median total daily protein intake was 43g (range 33−76g) daily, or 2.3g/kg/day (range 1-3.6g/kg/day). It provided a median intake of 201% (range 133−296%) of the dietary reference value for protein (DH, 1991).

Timing of protein substitute intake

Although patients were advised to consume the protein substitute in equal amounts three times daily, at set times with meals, actual practices varied considerably. For at least 80% of the study, only 37% of the patients took the protein substitute three times daily at mealtimes, 42% took it twice daily at breakfast and with the evening meal, and 21% took all the protein substitute in the morning or in the evening. Twenty six percent of these patients varied the timing for a small number of the study days.

e

Energy intake

Patients had a mean (SD, range) energy intake of 105% (17%, 77–178%) of the DH (1991)

estimated average requirements. The median intake per kg was 80 kcal

(range 25–157 kcal/kg/day) which decreased with age (p <0.0001) (Fig 2.4). The median

day to day variability was only 16% (range 11–30%) (Fig 2.5). The energy intake was

distributed throughout the day as follows: 29% breakfast and pre-midday; 36% lunch and

pre-evening meal and 35% evening meal and bedtime. The five chief dietary energy sources

were crisps, low protein bread, cereal, liquid Duocal (Scientific Hospital Supplies),

margarine and butter. Twenty seven percent (range 7–58%) of the energy was provided by

the protein substitute.

The mean energy intake of each of the subjects correlated positively (r = 0.4404) with excess

protein intake from free foods, i.e. patients who had a better appetite and consumed more

energy from free foods, had a higher excess natural protein intake. This did not reach

statistical significance (p = 0.0591) (Fig 2.6). Furthermore, there was a weak negative

correlation (r = -0.309) between timing of protein substitute intake and total energy intake.

The total energy intake appeared to be lower when patients had a higher proportion of their

daily protein substitute before the evening meal. This did not reach statistical significance

(Fig 2.7).

Fat intake

The overall fat intake was low. The median daily intake was 44g (range 18-91g) or

1.9g/kg/day (range 0.5–6g/kg/day). It provided a median of only 23% (range 16–35%) of

the energy intake. Day to day fat intake varied more widely than energy, with a median

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the energy intake. Day to day fat intake varied more widely than energy, with a median

coefficient of variation of 30% (range 17–50%) (Fig 2.8). The daily fat intake was distributed as follows: breakfast and pre-midday 29%, lunch and pre-evening meal 42%; evening meal and bedtime 32%. The five most common fat sources in the diet were crisps, butter, margarine, liquid Duocal (Scientific Hospital Supplies) and Calogen (Scientific Hospital Supplies). The protein substitute contributed almost no dietary fat, with the exception of one child who took part of his protein substitute requirements from the infant product, *XP* Analog (Scientific Hospital Supplies).

Carbohydrate

The carbohydrate intake was high. The median daily intake was 249g (range 150–4238) daily, or 13,2g/kg/day (range 4-23g/kg/day). It provided 59% (range 53–69%) of the energy intake. The median daily coefficient of variation was only 17% (range 10-29%)(Fig 2.9). It was fairly evenly distributed throughout the day; 30% breakfast and pre-midday; 34% lunch and pre-evening meal; and 36% evening-meal and bedtime. The protein substitute supplied over a quarter of the carbohydrate intake: median intake of 27% (range 0-64%). Other important dietary carbohydrate sources include: low protein bread, breakfast cereals, super soluble Maxijul (Scientific Hospital Supplies), potatoes and crisps.

Alcohol

Only one patient consumed alcohol on two occasions. It contributed a mean of 1% (SD 3.2) of total energy intake for this patient.

2.4.2 Blood phenylalanine results

Variation between plasma phenylalanine concentrations in early morning and late afternoon blood specimens

For some patients, there was considerable variability between day-to-day early morning and late afternoon plasma phenylalanine concentrations: plasma phenylalanine, mean a.m. 282 μmol/l (SD 162) (Fig 2.10); mean p.m. 243 μmol/l (SD 184) (Fig 2.11). Although there was a Significant decrease between mean early morning and late afternoon plasma phenylalanine concentrations ($p = 0.012$) there was a widespread variation in the change between early morning and late afternoon concentrations: mean plasma phenylalanine change -40 μmol/l (SD 62) (Fig 2.12). Thirty per cent (n = 107) of all morning plasma phenylalanine concentrations increased between early morning and late afternoon.

Effect of dietary phenylalanine intake from freely allowed foods on plasma phenylalanine concentrations

For almost all patients there was no significant within-subject correlation between excess natural protein intake or total protein intake and (1) pre-breakfast or pre-evening meal plasma phenylalanine concentrations; or (2) daily change between pre-breakfast and pre-evening meal plasma phenylalanine concentrations. Similarly there was no significant correlation between excess natural protein intake on the previous day and plasma phenylalanine concentration on the following morning (Table 2.2)

Effect of energy intake on plasma phenylalanine concentrations

For the majority of patients there was no significant correlation between energy intake and (1) pre-breakfast or pre-evening meal plasma phenylalanine concentrations; or (2) the difference between pre-breakfast and pre-evening meal plasma phenylalanine concentrations.

Table 2.2. Effect of excess percentage protein intake from freely allowed foods on

a) a.m. plasma phenylalanine concentrations.
b) p.m. plasma phenylalanine concentrations.
c) a.m.-p.m. change in plasma phenylalanine concentrations
d) following a.m. plasma phenylalanine concentrations.

Patient No.	a) Correlation coefficient for excess percentage protein intake and morning (a.m.) plasma phenylalanine concentrations	p	b) Correlation coefficient for excess percentage protein intake and evening (p.m.) plasma phenylalanine concentrations	p	c) Correlation coefficient for excess percentage protein intake and a.m. – p.m. change in plasma phenylalanine concentrations	p	d) Correlation coefficient for excess protein intake and following a.m. plasma phenylalanine concentrations	p
1	-0.2375	NS	-0.3763	NS	-0.02505	NS	-	-
2	0.3053	NS	-0.2243	NS	-0.5940	0.0251	0.6755	NS
3	0.3093	NS	0.2517	NS	-0.05609	NS	0.4350	NS
4	0.7895	<0.0001	0.7862	<0.0001	0.1021	NS	0.7904	NS
5	0.2199	NS	0.1367	NS	-0.1489	NS	0.2441	NS
6	-0.1765	NS	-0.04526	NS	0.02144	NS	-0.3102	NS
7	0.08510	NS	0.1009	NS	-0.01535	NS	0.3650	NS
8	0.1675	NS	0.1743	NS	-0.2978	NS	-0.1988	NS
9	-0.7177	0.0086	-0.4929	NS	0.3554	NS	-0.2580	NS
10	0.1987	NS	0.3553	NS	0.2704	NS	0.1712	NS
11	-0.2342	NS	0.1063	NS	0.3116	NS	0.1003	NS
12	0.3699	NS	-0.3184	NS	0.2557	NS	-0.1768	NS
13	0.1063	NS	0.1842	NS	0.1963	NS	-0.1755	NS
14	0.4433	0.0441	0.1005	NS	-0.4583	0.0367	0.5657	NS
15	0.5221	0.0219	0.07145	NS	-0.04315	NS	0.0569	NS
16	0.1742	NS	0.03924	NS	-0.1478	NS	0.1785	NS
17	0.1094	NS	-0.09981	NS	-0.2308	NS	0.0421	NS
18	-0.3793	NS	-0.2848	NS	-0.1698	NS	-0.3548	NS
19	0.1584	NS	0.05625	NS	-0.01373	NS	0.1888	NS

Nor was the previous day's energy intake significantly related to the following morning's plasma phenylalanine concentration (Table 2.3).

Effect of timing of protein substitute on plasma phenylalanine concentrations

The timing of protein substitute ingestion had a major impact on changes in plasma phenylalanine concentration. There was a strong negative correlation between the amount of protein substitute taken by the time of the evening meal and the change in plasma phenylalanine concentrations during the day ($r = 0.941$, $p < 0.0001$) (Fig 2.13). The more protein substitute taken early in the day, the greater the fall in plasma phenylalanine concentrations during the course of that day. On the basis of recommendations that children with PKU take the protein substitute three times daily with meals, it can be expected that patients should have taken at least 65% of it by the evening meal. In children who had taken more than 65% of their protein substitute by the time of their evening meal, almost half (49%) of pre-evening meal plasma phenylalanine concentrations were less than 100μmol/l. Conversely, the less the protein substitute taken before the evening meal, the higher the increase in plasma phenylalanine concentrations during the day.

There was a positive correlation between the quantity of protein substitute taken by the time of the evening meal and the overnight change in plasma phenylalanine concentrations which subsequently took place between the pre-evening meal and the following pre-breakfast samples ($r = 0.8724$, $p < 0.0001$) (Fig 2.14).

Table 2.3 Effect of % energy intake compared with EAR on:

a) a.m. plasma phenylalanine concentrations
b) p.m. plasma phenylalanine concentrations
c) a.m.-p.m. change in plasma phenylalanine concentrations
d) following a.m. plasma phenylalanine concentrations

Patient No.	a) Correlation coefficient of energy intake and a.m. plasma phenylalanine concentrations	p	b) Correlation coefficient for energy intake and p.m. plasma phenylalanine concentrations	p	c) Correlation coefficient of energy intake and a.m. – p.m. change in plasma phenylalanine concentrations	p	d) Correlation coefficient of energy intake and following a.m. plasma phenylalanine concentrations	p
1	-0.1276	NS	0.05137	NS	0.226	NS	-	NS
2	-0.3041	NS	-0.6798	NS	0.4304	NS	0.1697	NS
3	0.3388	NS	0.4050	NS	0.2109	NS	0.3145	NS
4	0.8473	0.0003	0.8700	<0.0001	-0.3314	NS	0.7920	NS
5	-0.01442	NS	-0.2566	NS	-0.4748	0.0256	0.01291	NS
6	-0.2808	NS	-0.09367	NS	-0.009833	NS	-0.4925	NS
7	0.3040	NS	-0.007492	NS	-0.3179	NS	0.1530	NS
8	0.4789	0.0281	-0.5664	NS	-0.3578	NS	0.3200	NS
9	0.07324	NS	-0.2326	NS	-0.6020	0.0384	-0.1917	NS
10	-0.1395	NS	-0.2642	NS	-0.2134	NS	-0.3673	NS
11	0.1068	NS	-0.1627	NS	-0.2059	NS	-01603	NS
12	-0.3467	NS	-0.4799	0.0376	-0.1384	NS	-0.3421	NS
13	-0.3404	NS	-0.3713	NS	-0.1714	NS	-0.5917	0.0427
14	0.02048	NS	-0.3227	NS	-0.5257	0.0144	0.2020	NS
15	0.05079	NS	0.2032	NS	0.4301	NS	0.4333	NS
16	-0.4065	NS	-0.4357	0.0484	0.004202	NS	-0.3462	NS
17	0.04952	NS	-0.003759	NS	-0.008319	NS	0.3353	NS
18	-0.5534	0.0093	-0.1997	NS	0.5838	0.005	-0.6636	0.0186
19	-0.2622	NS	-0.3569	NS	-0.3004	NS	-0.7017	0.0161

Multiple regression analysis

Using multiple regression analysis, the effect of change in morning to evening plasma

phenylalanine concentrations of all 3 variable factors i.e.: (1) timing of protein substitute intake,

(2) dietary phenylalanine intake from freely allowed foods and (3) energy intake were

compared. (Table 2.4)

Table 2.4

Multiple regression analysis: Factors affecting variability in blood phenylalanine concentrations

Variable factors	Regression coefficient	SD	t – ratio	p
Timing of protein substitute intake	- 2.8340	0.2581	- 10.98	<0.001
Excess natural protein from freely allowed foods	- 0.1876	0.1610	- 1.17	0.262
Total energy intake (% EAR)	- 0.1431	0.2192	- 0.65	0.524

R-squared = **89.7%**   R-squared (adjusted = **87.6%**)

This confirmed that the only factor, which significantly correlated with change in plasma

phenylalanine concentrations between a.m. and p.m., was timing of protein substitute intake.

Using stepwise regression, when the effect of energy intake was removed from the equation, the

effect of dietary phenylalanine from freely allowed food changed only slightly (regression

coefficient = -0.2258; **SD 0.1473; t** − ratio **-1.53;** p= 0.145).

2.5 Summary

This study shows large diurnal fluctuations ir. plasma phenylalanine concentrations in a group of

patients who had been carefully selected on the basis that they maintained routinely taken

plasma phenylalanine concentrations within ranges recommended by the MRC working group.

Furthermore, it shows that blood samples pre-breakfast plasma phenylalanine concentrations

were not always higher than in the evening. The MRC report recommended that blood

specimens should be taken in the early morning when plasma concentrations were considered **to**

be at a peak.

This study also shows that 'free' low protein foods contribute a significant quantity of additional

excess protein and phenylalanine. It demonstrates a large day-:+day   variation **in** natural protein

intake. However, plasma phenylalanine concentrations were strongly influenced by the timing

of the protein substitute, but not by total energy or excess natural protein intake from 'freely'

allowed foods. Patients who had taken 65% or more of their protein substitute by the time of

their evening meal showed a fall in plasma phenylalanine concentration during that day. In

contrast, patients who delayed most or all of their protein substitute until after their evening

meal showed a **rise** in plasma phenylalanine concentration during the day. Patients who took

their protein substitute in two doses evenly distributed between morning and evening showed

little variation in their plasma phenylalanine concentrations. **For** the study group overall, there

was significantly greater variability in evening plasma phenylalanine concentrations than for

morning concentrations.

Fig 2.1   **Daily protein intake (%) in excess of allocated phenylalanine exchanges for individual subjects**
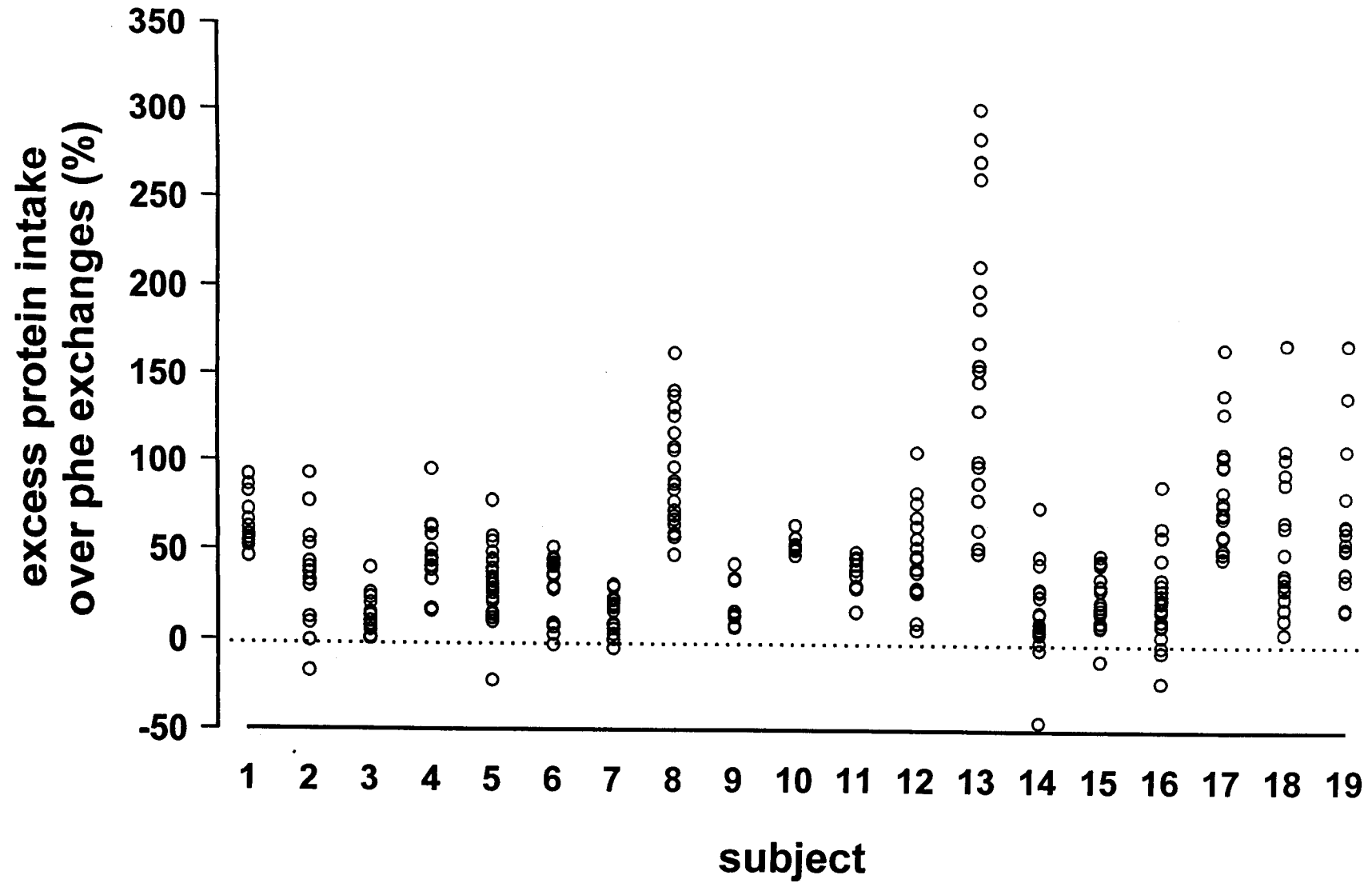
Fig 2.2   Correlation between protein and phenylalanine intake

r=0.9745
p<0.0001
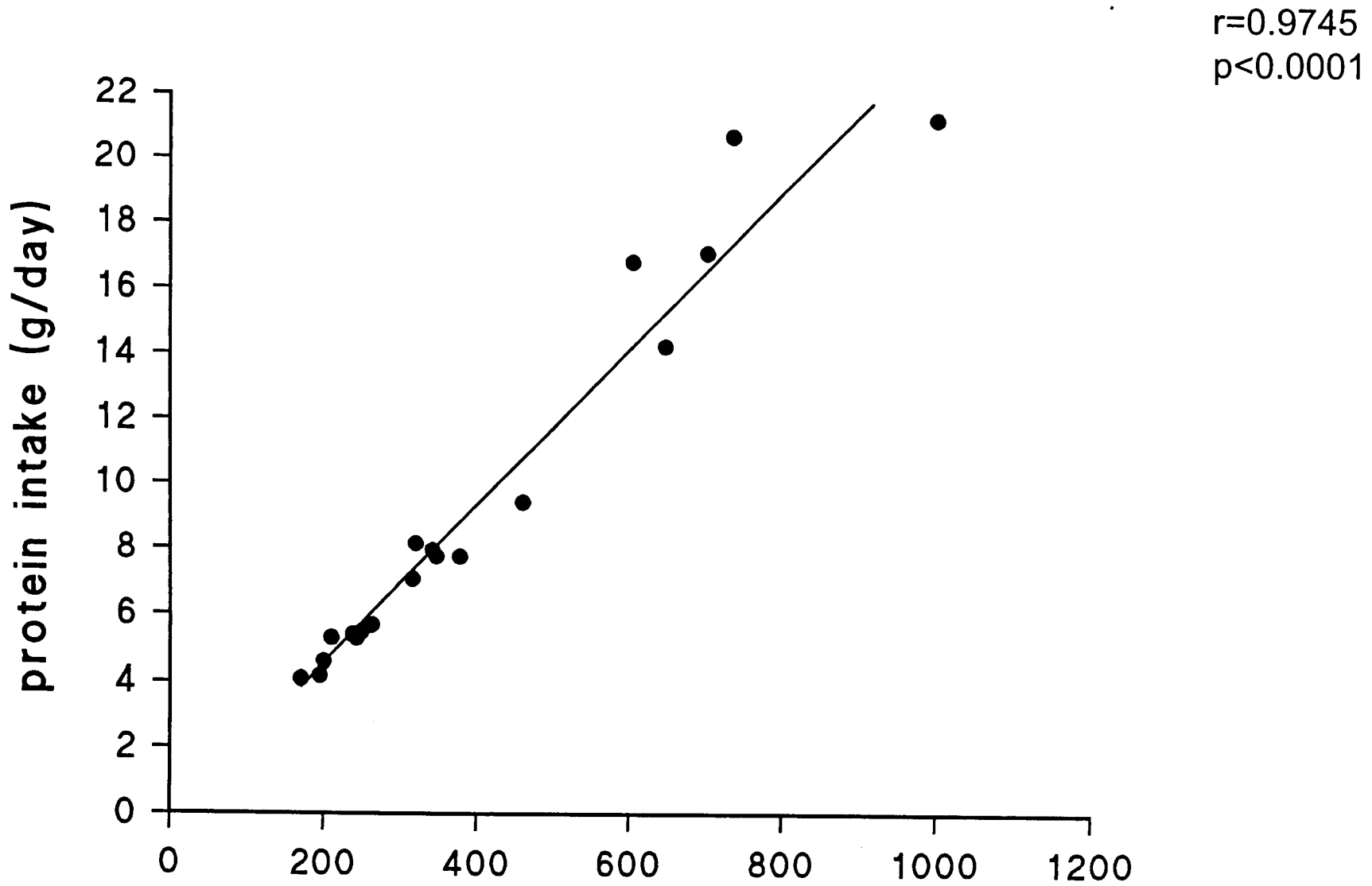
Fig 2.3   Total daily phenylalanine intake for individual subjects
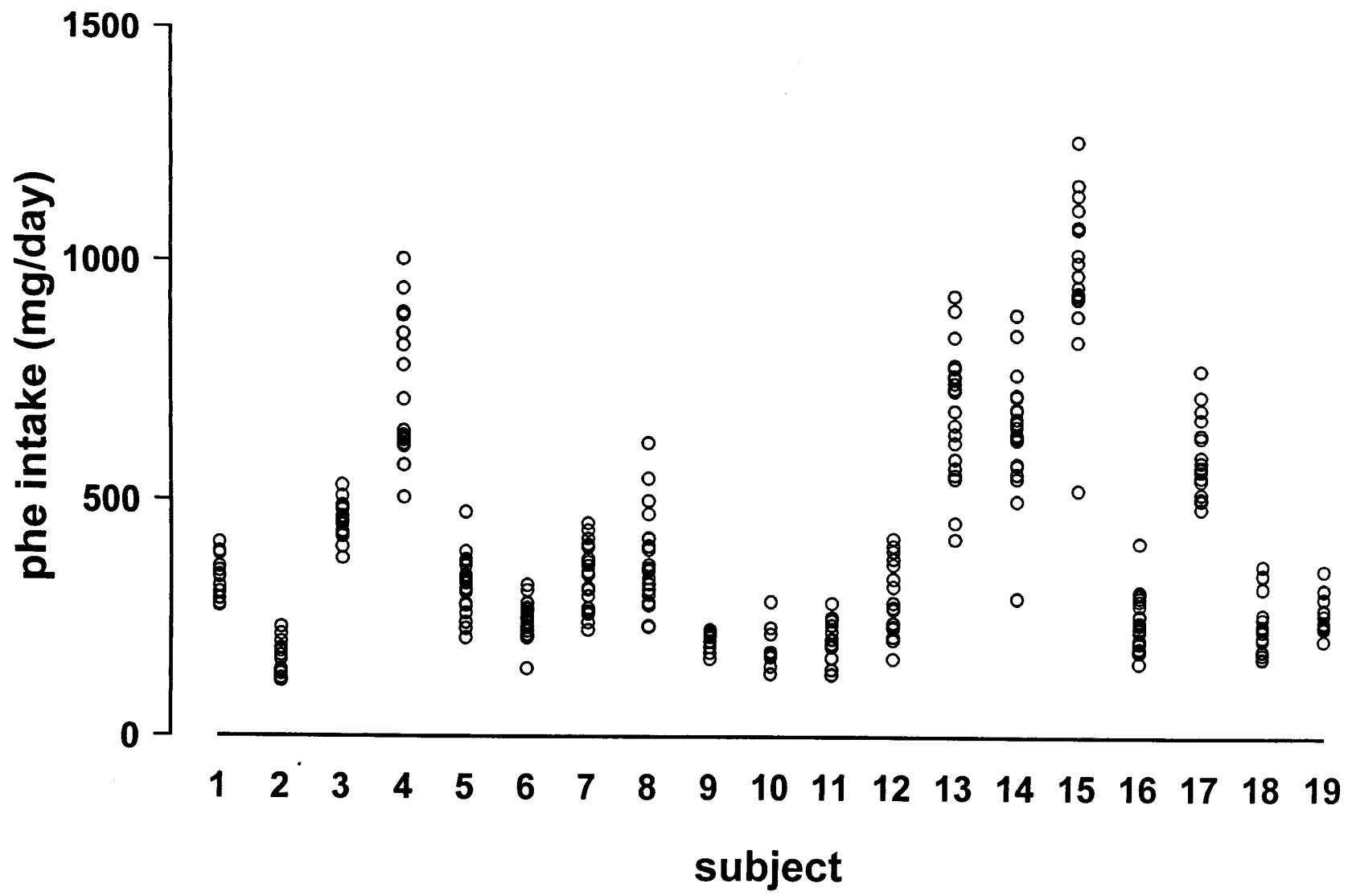
Fig 2.4 **Correlation between dietary energy intake (kcal/kg/day) and age**

r=-0.8121
p<0.0001
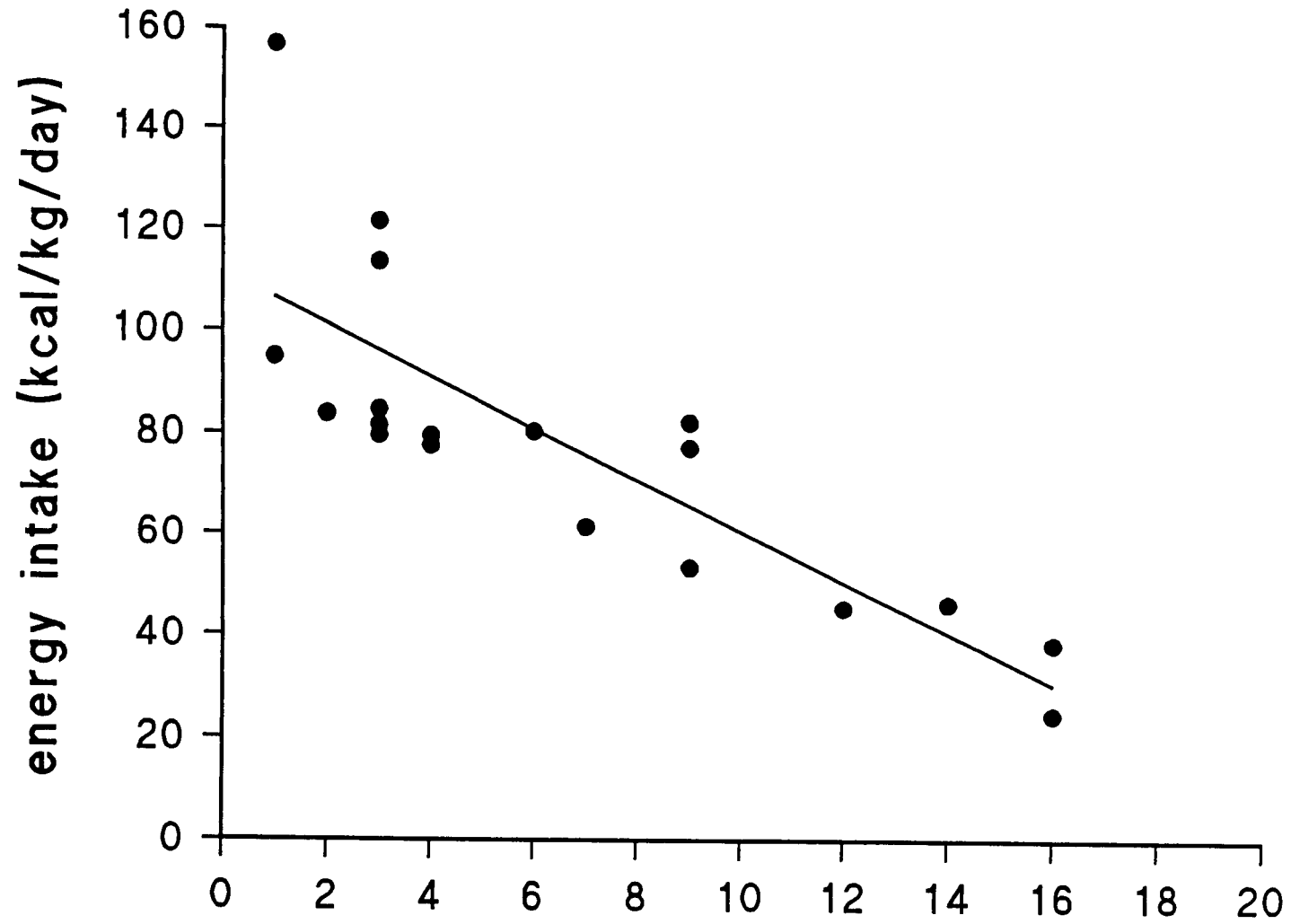
Fig 2.5

Total daily energy intake expressed as a percentage of EAR for individual subjects
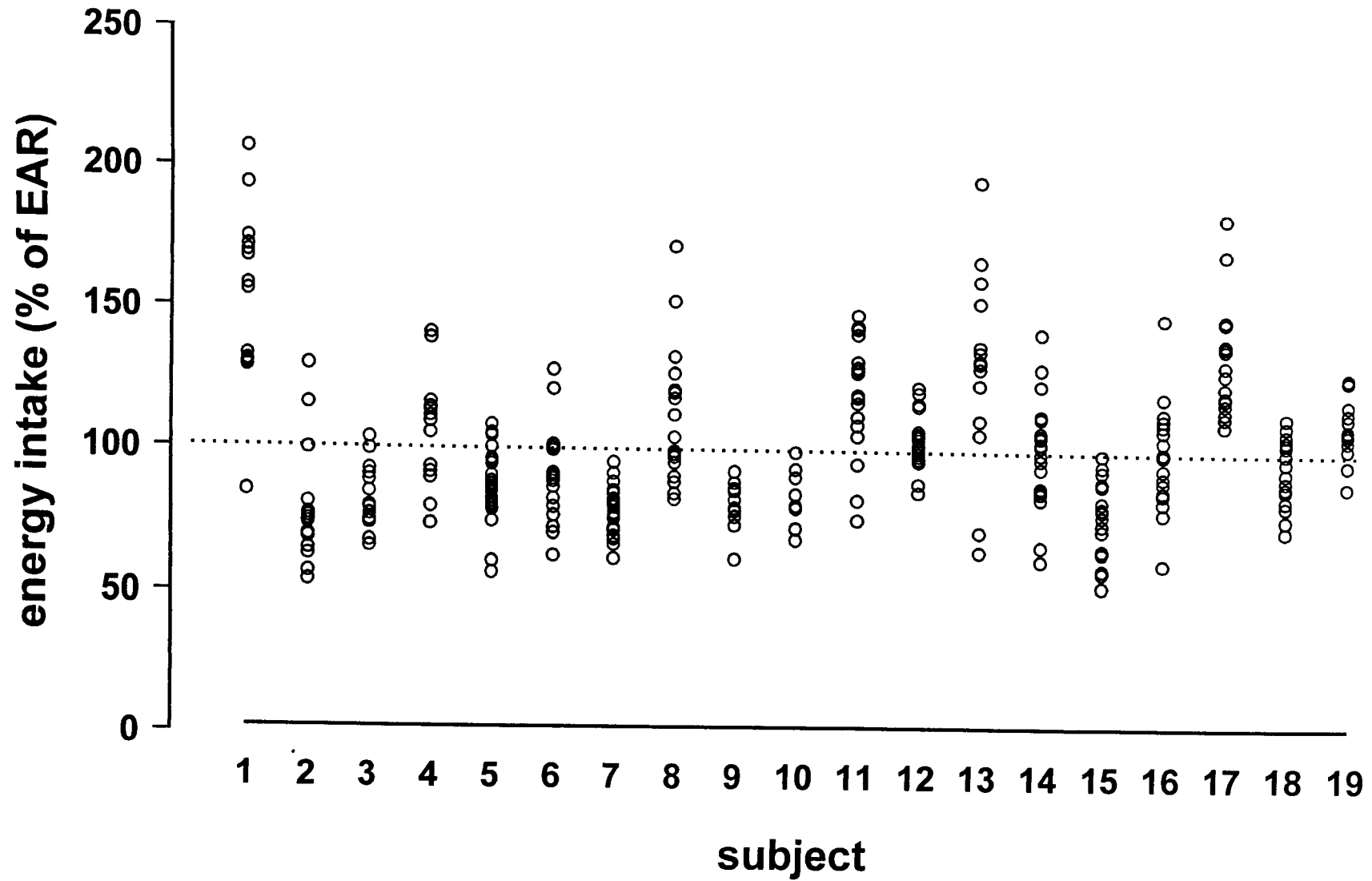
Fig 2.6 Correlation between energy intake expressed as % of EAR and excess natural protein intake over allocated phenylalanine exchanges

$r=0.4404$
$p=0.0591$
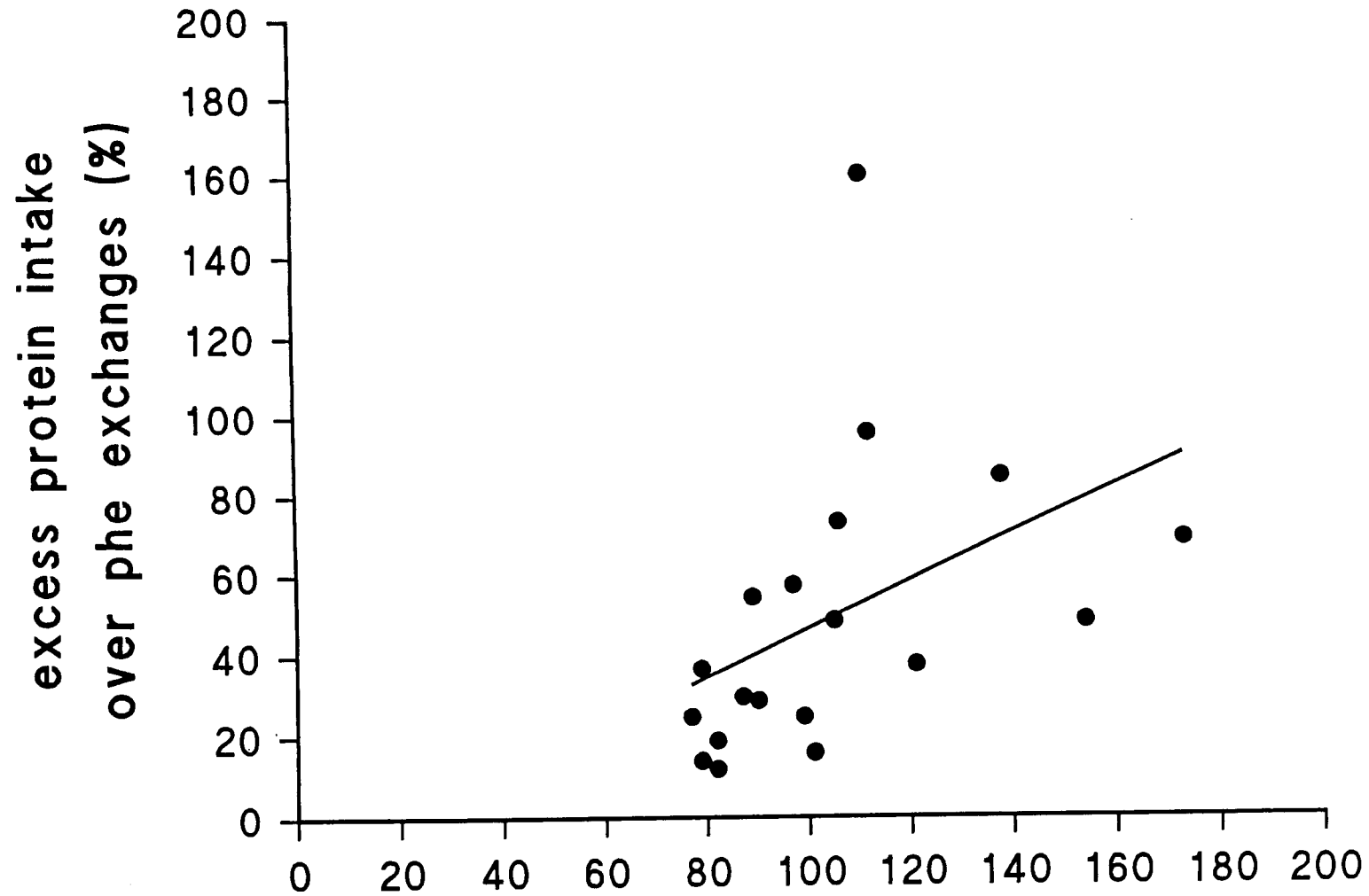
Fig 2.7

Correlation between % of protein substitute intake before evening meal and energy intake (expressed as % of EAR)

r=0.3174
p=0.1855

energy intake (% of EAR)

mean % protein substitute taken by evening meal
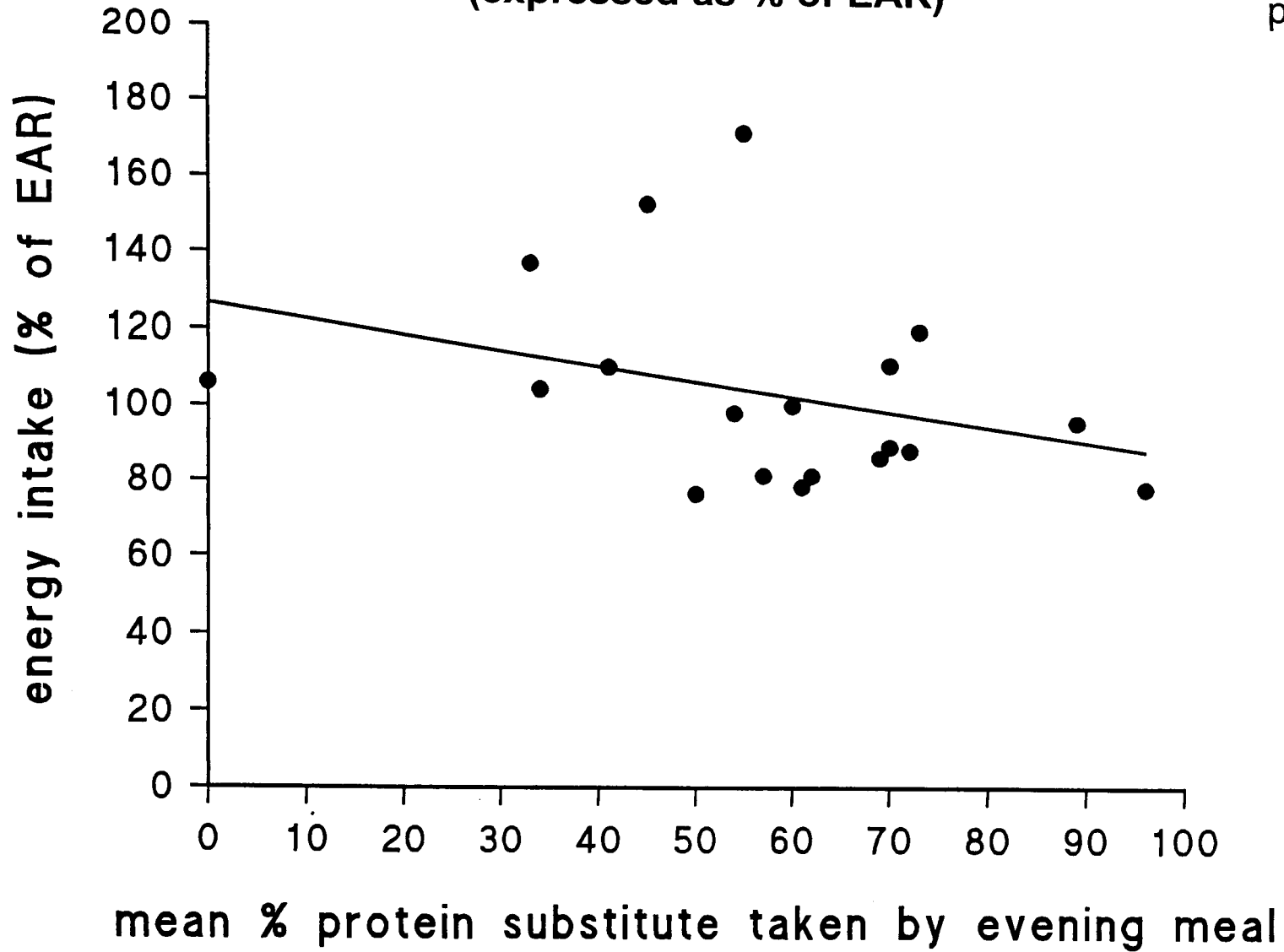
Fig 2.8   Total daily dietary fat intake for individual subjects
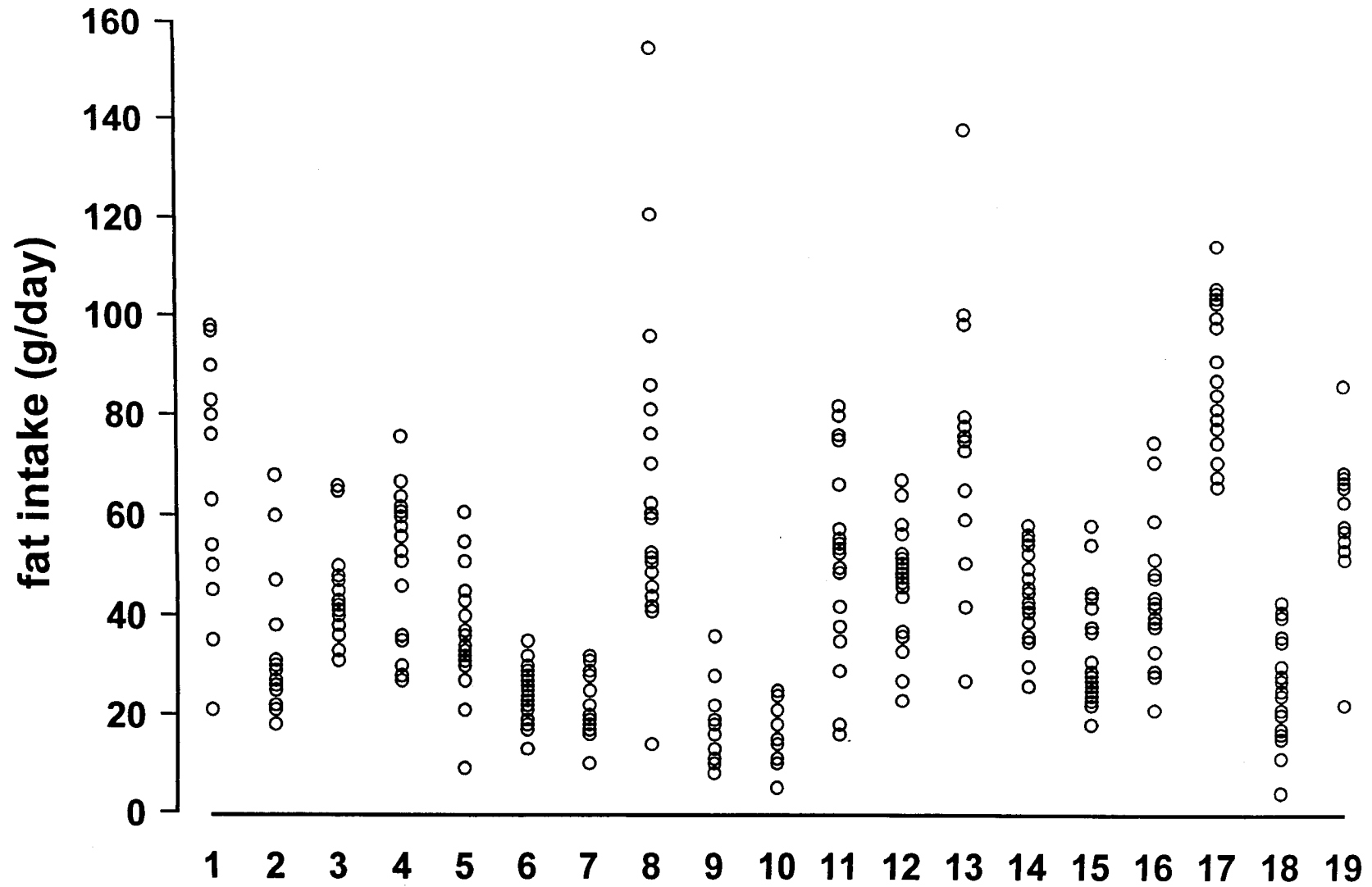
Fig 2.9

Total daily dietary carbohydrate intake for individual subjects
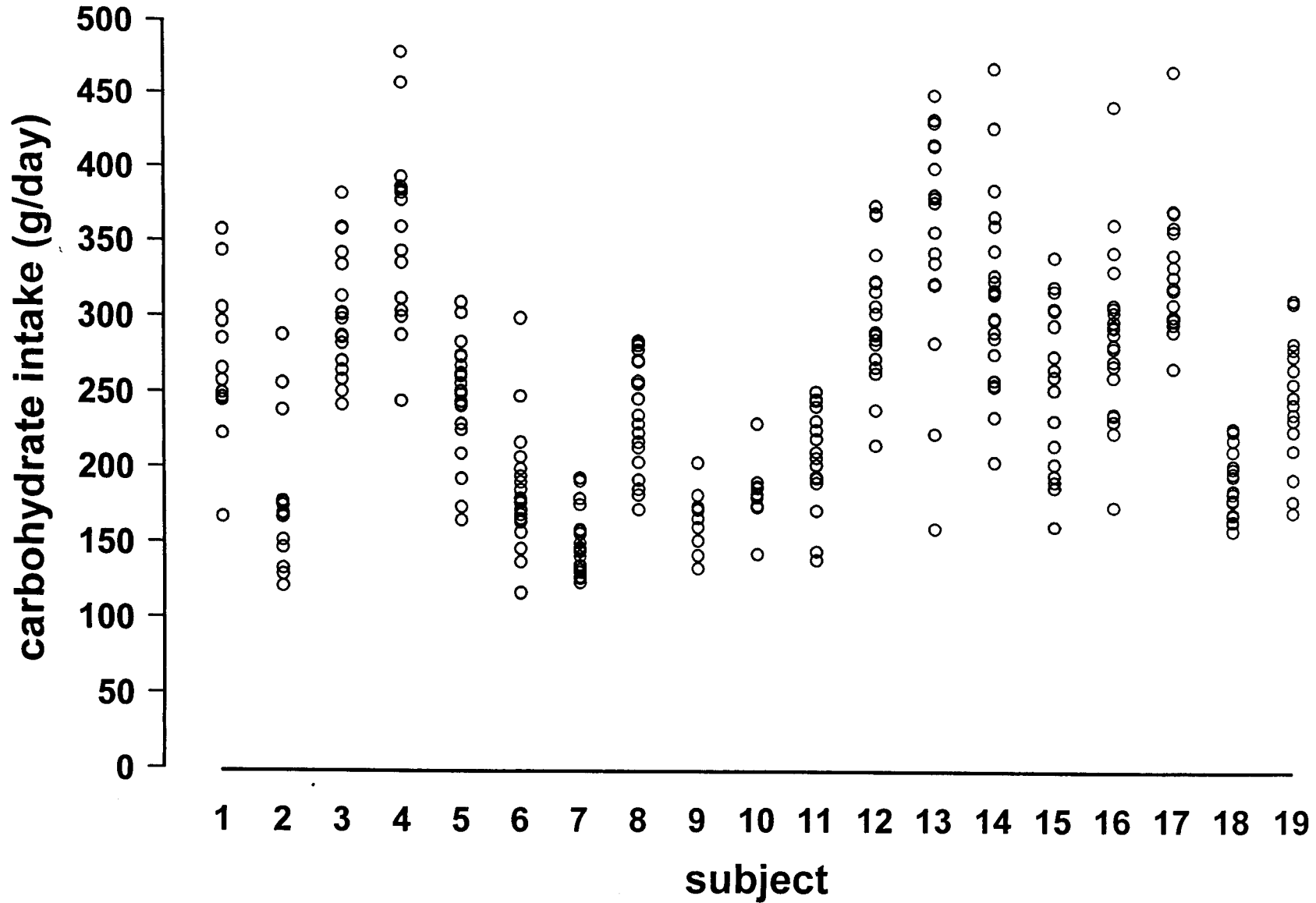
Fig 2.10 **All morning plasma phenylalanine concentrations for individual subjects**
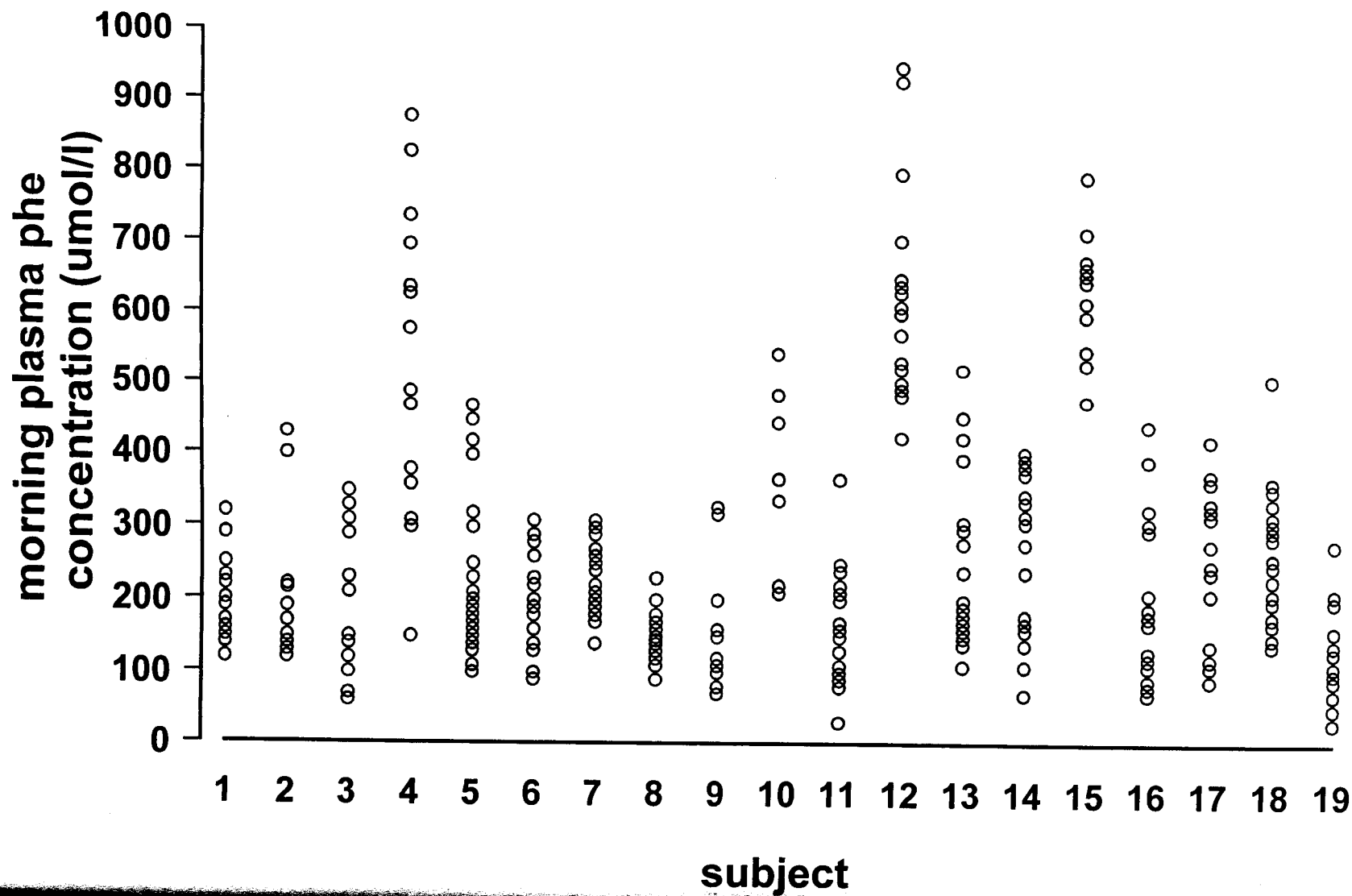
Fig 2.11   All evening plasma phenylalanine concentrations for individual subjects
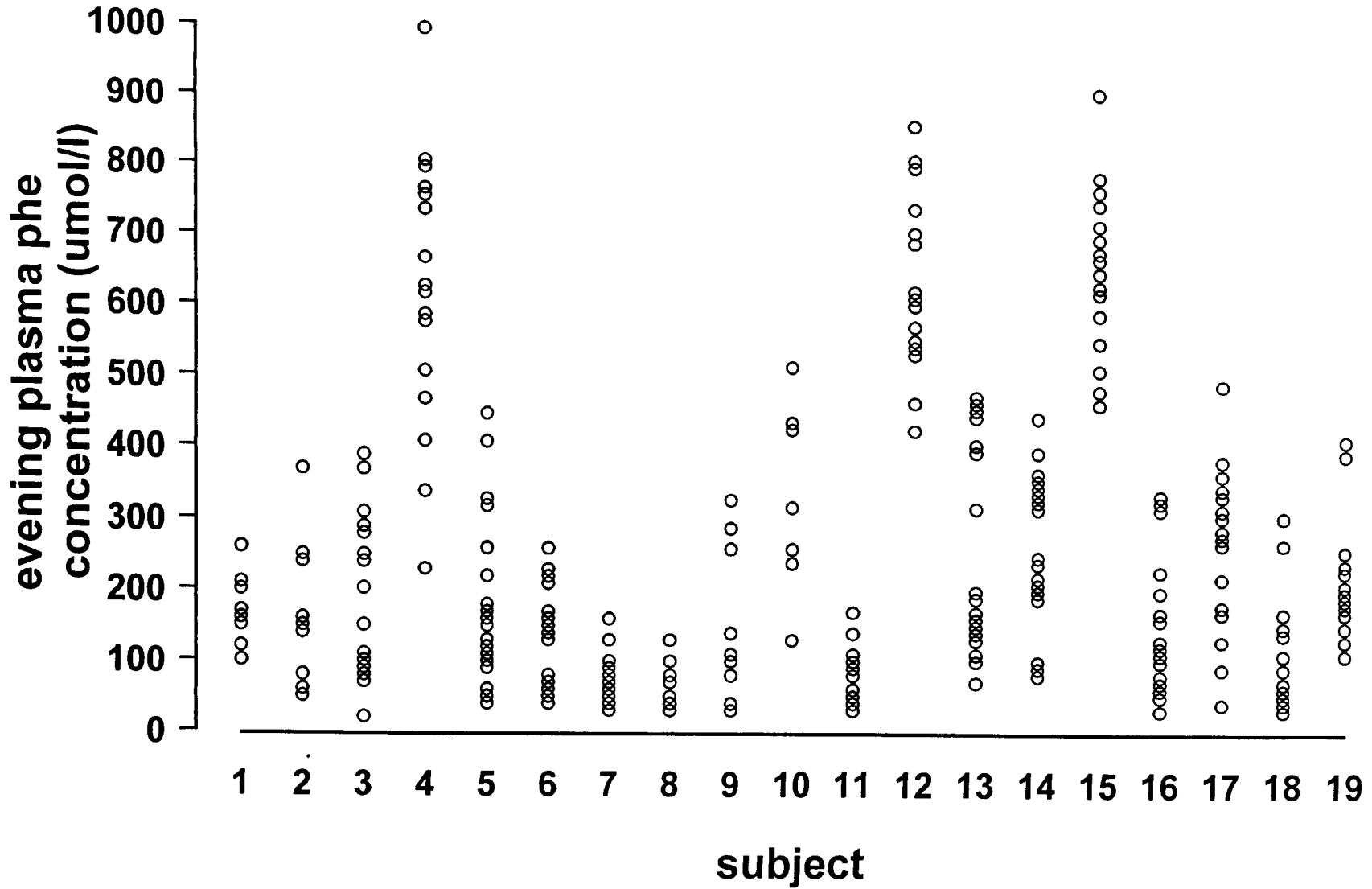
Fig 2.12   Change in plasma phenylalanine concentrations between morning and evening for individual subjects
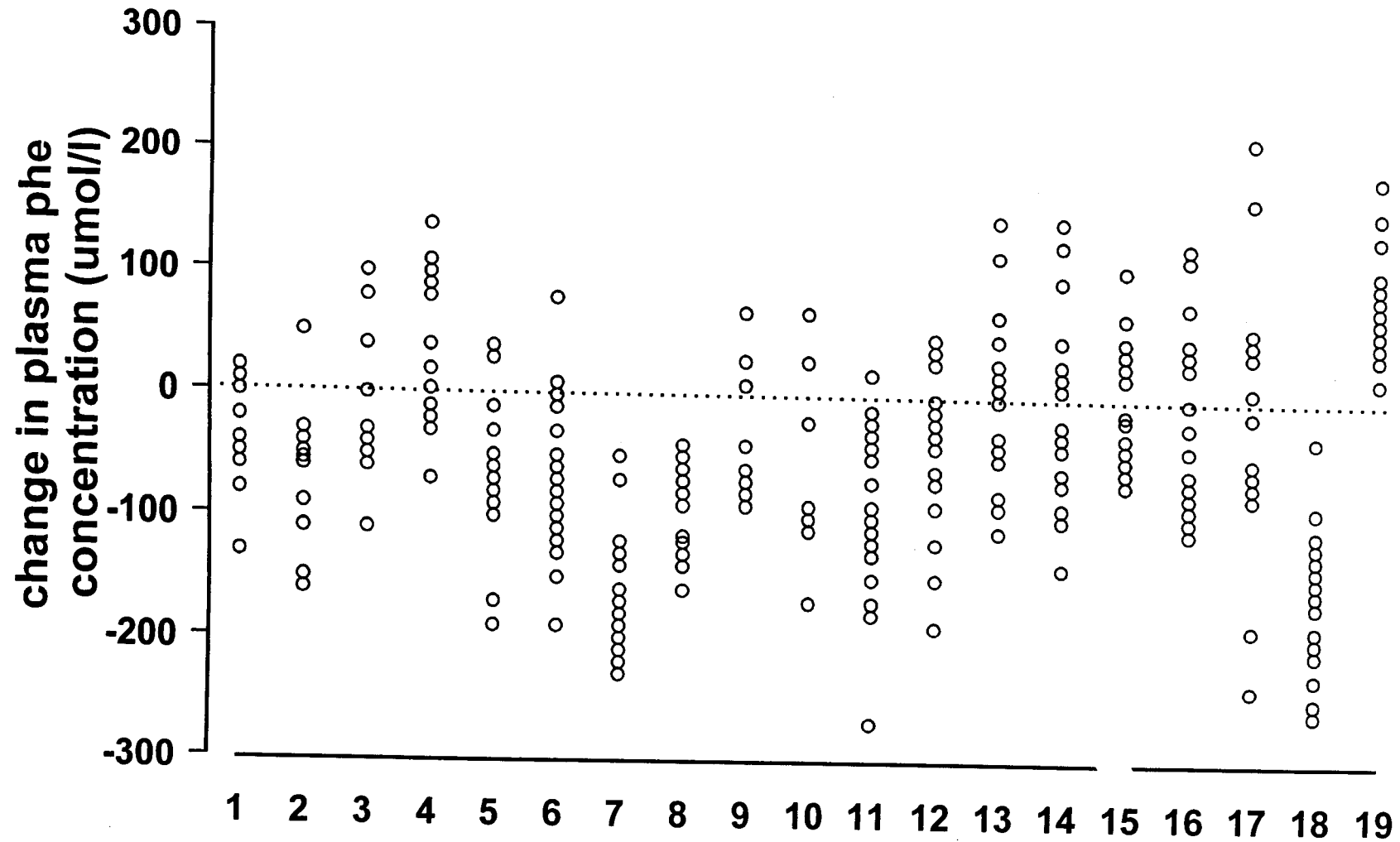
Fig 2.13 Correlation between mean protein substitute intake taken before evening meal and change between morning to evening plasma phenylalanine concentrations (I=SEM)

r=-0.9413
p<0.0001

mean change between am and pm blood phe concentration
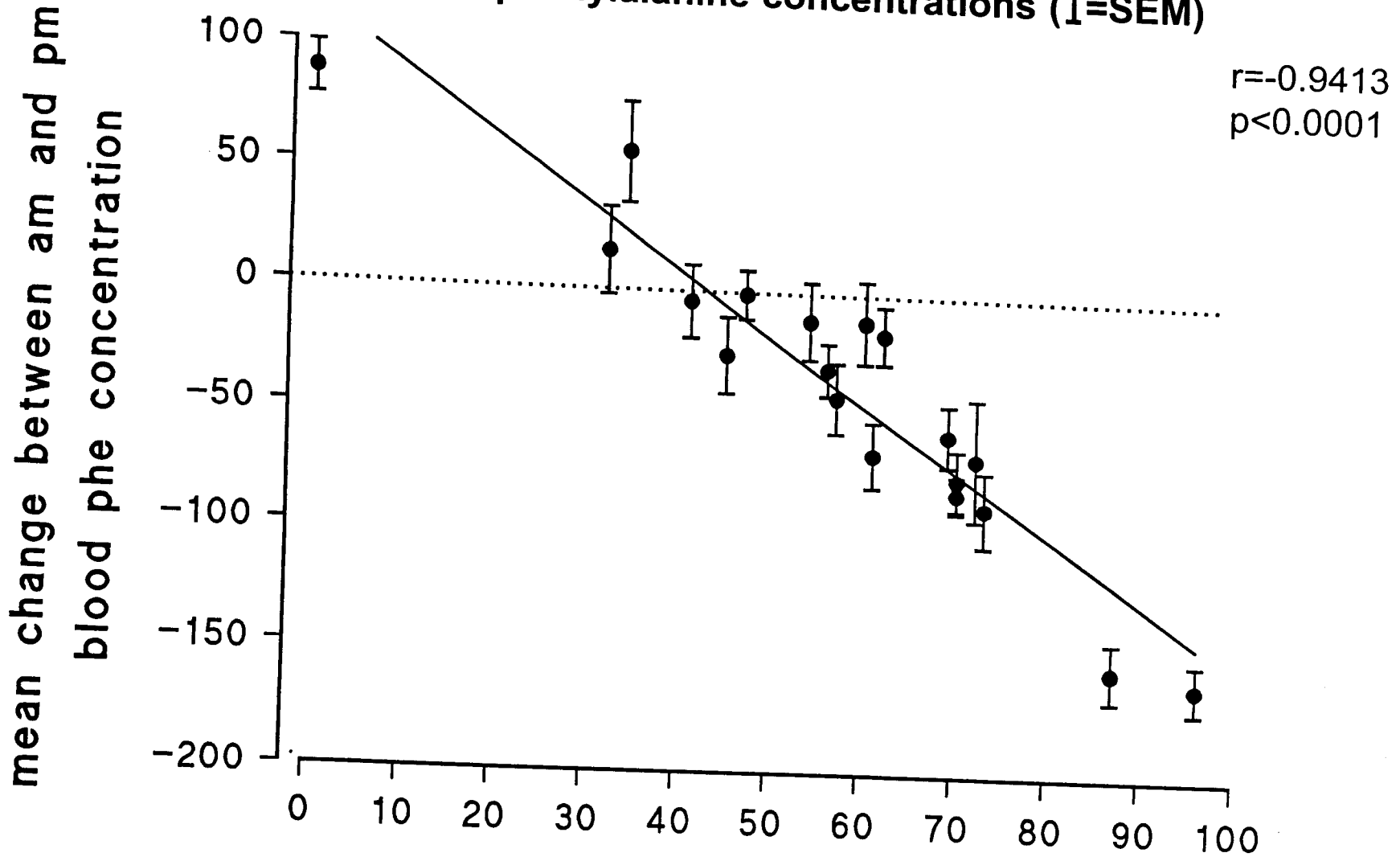
Fig 2.14 Correlation between mean protein substitute intake taken before evening meal and change between evening to morning plasma phenylalanine concentrations (I=SEM)
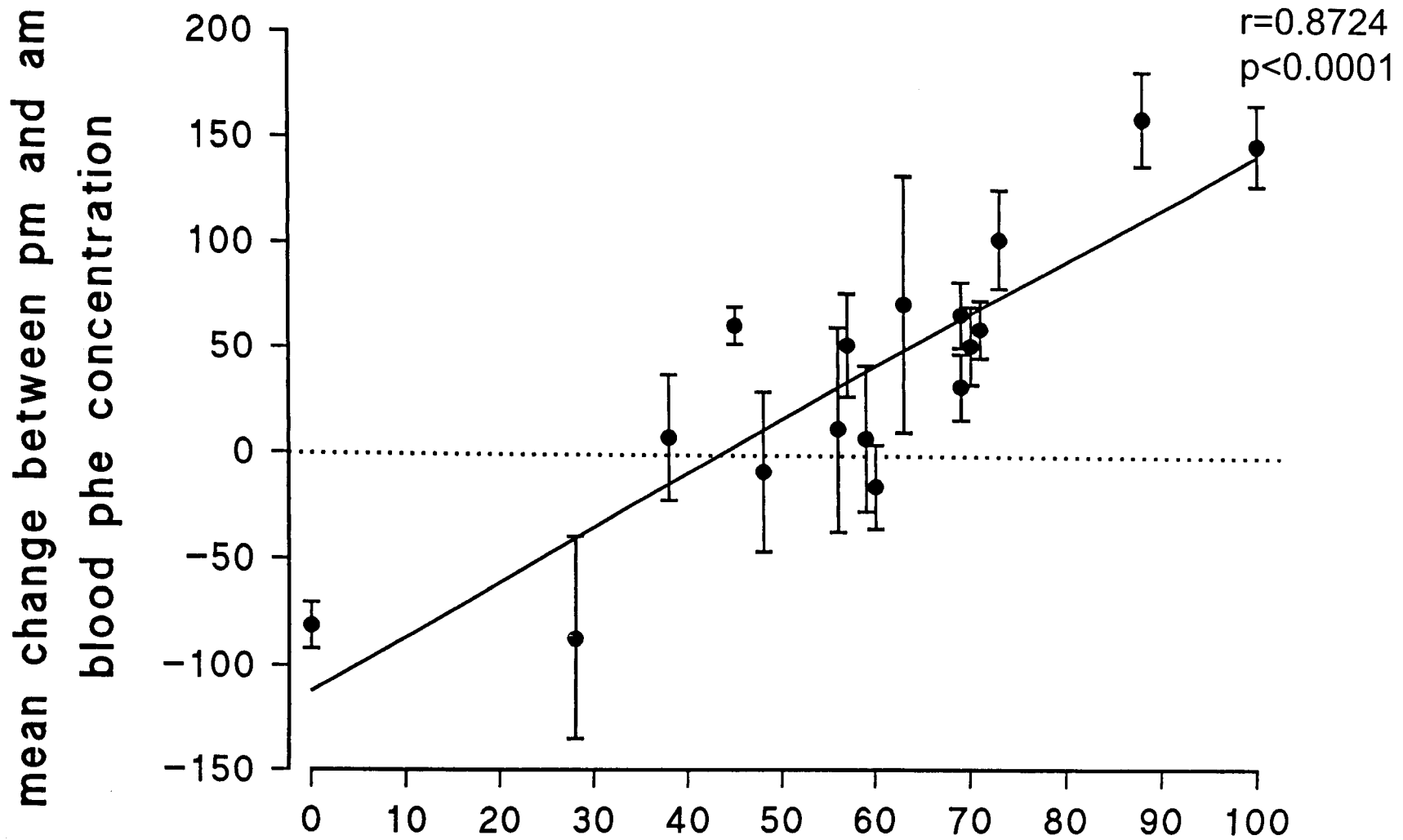
Chapter 3

A prospective, cross-over trial assessing the impact of new potato exchange allowances on blood phenylalanine concentrations in PKU

3.1 Introduction

potato is one of the most commonly used 50 mg phenylalanine exchanges in PKU. Some children have 20–50% of their daily phenylalanine from this source. Traditionally, phenylalanine exchanges are extrapolated from total protein content rather than the phenylalanine content of foods. It is assumed that 1g protein yields 50 mg phenylalanine (Barnes, **1994**). In **1996,** the phenylalanine content of a number of varieties of potato prepared in different ways, were analysed (Weetch, **1997**). Overall phenylalanine content was much lower than in previously published reports. On average, one gram of protein yielded only **28** mg phenylalanine for potatoes. **As** a consequence, the phenylalanine exchanges for potato have been revised and are now based specifically on phenylalanine data. This has resulted in the quantity of potato allocated for phenylalanine being almost doubled.

As potato is such a staple food in the PKU diet, it is possible that allowing the higher amounts of potato for phenylalanine exchanges may change blood phenylalanine concentrations. We therefore compared plasma phenylalanine concentrations in a group of subjects following the use of potato exchanges based on old or revised criteria.

3.2 Subjects and methods

3.2.1 Subjects

Sixteen subjects (**1**5 Caucasian, 1 Afro-Caribbean) were recruited into the study. There were **14** girls and **2** boys with a mean age of 7 years (**1-24** years). All had classical PKU. There were four inclusion criteria: **i**) maintenance of at least 70% plasma phenylalanine

concentrations (weekly, fortnightly or monthly according to age group) within ranges recommended by the MRC Working Group (1993) in the 6 months before entering the study; 2) age over 1 year; 3) ability to eat prescribed quantity of potato; and 4) demonstrable parental ability to take skin puncture blood specimens at home.

The median number of 50 mg phenylalanine exchanges was 6 daily (range 4 to 16). The following amounts of total protein from protein substitute and phenylalanine exchanges were allocated: age <2 years: 3.0g/kg/day; 2 − 5 years: 2.5g/kg/day; 6 − 10 years: 2.0g/kg/day; over 11 years of age: I.5g/kg/day. Patients were given their usual brand of protein substitute. This was taken either as a paste or drink. Nine patients used XP Maxamaid (Scientific Hospital Supplies), five used Phlexy 10 Drink Mix (Scientific Hospital Supplies), one used Aminogran Food Supplement (UCB Pharma) and one used XP Maxamum (Scientific Hospital Supplies).

This study was approved by the Committee on Medical Ethics of South Birmingham Health Authority. Informed consent was obtained from all parents and competent patients.

3.2.2 Study design

This was an 8-week, open, prospective crossover study conducted at home (Fig 3.1). During the first 3 weeks, subjects ate at minimum of one potato 50 mg phenylalanine exchange daily using phenylalanine data extrapolated from protein content (Table 3.1). During weeks 4 to 8, subjects ate a minimum of one potato 50 mg phenylalanine exchange daily using the new phenylalanine content. Over the last 3 days on weeks 1, 3, 6 and 8, twice-daily serial skin

Fig 3.1   **Study design of a 8 week, cross-over study comparing the effect of potato exchanges calculated from either protein content or direct phenylalanine measurement**

Weeks 1 – 3	Weeks 4 – 8
Daily potato phenylalanine exchanges eaten based on assumed phenylalanine content derived from protein content	Daily potato phenylalanine exchanges eaten based on direct phenylalanine content

Week 1
Days 5 – 7

- Twice-daily blood samples for phenylalanine

- Constant menu

Week 3
Days 5 – 7

- Twice-daily blood samples for phenylalanine

- Constant menu

Week 6
Days 5 – 7

- Twice-daily blood samples for phenylalanine

- Constant menu

Week 8
Days 5 – 7

- Twice-daily blood samples for phenylalanine

- Constant menu

blood specimens were collected at the same time each day, standardised for each patient, but always pre-breakfast and pre-evening meal.

Table 3.1 Estimated and directly measured phenylalanine content of potatoes

Type of potatoes	Phenylalanine mg/l00g[1]	Protein g/100g[2]	50 mg phenylalanine exchange weight (g) based on protein content'	Average 50 mg phenylalanine exchange weight (g) based on phenylalanine content[4]
Boiled potatoes	44	1.8	55	80
Nicola boiled potatoes	70	1.8	55	80
Mashed potatoes	43	1.8	55	80
Jacket potato with **skin**	62	3.9	25	80
Chips	108	3.2	25	45
Roast potato	88	2.9	35	55

1. Weetch *et al,* **1997**
2. Holland *et al,* **1991**
3. **NSPKU, 1994**
4. **NSPKU, 1998**

On the same days that the blood specimens were taken, subjects followed the same standard menu which was individualised for each patient. This was a repeat of the food intake which had been eaten on the last three days of week 1, but with the addition of the increase in weight of the potato exchanges, based on the new phenylalanine content, during weeks **6** and **8.** All food intake was weighed and recorded on blood sampling days.

Assessment of plasma phenylalanine concentrations

parents or subjects took heel or thumb skin puncture blood specimens at home, and immediately posted them to the hospital. Blood collection technique had been taught and competence assessed by a specialist nurse. All blood specimens were centrifuged upon receipt and resulting plasma samples stored at -20°C until analysed. Plasma phenylalanine concentrations were measured by HPLC as previously described.

Assessment *of* dietary intake

Throughout the entire study, parents or subjects kept a daily diary of all potato exchanges eaten. After week 1, on blood sampling days, the only food and drinks parents or carers were instructed they could **vary** were very low phenylalanine foods (containing <0.3g/100g of protein), such as sweets and squash drinks. They were instructed to keep to standard times for administration of protein substitute on these days. All food and drink consumed was weighed using Salter scales (accurate to 5g), about which parents or older subjected had been instructed. **Only** food and drink actually consumed was recorded; any remaining food was deducted from the start weight.

Protein and nutritional analysis of food was calculated using the Microdiet computer programme based on *McCance & Widdowson's "The Composition of Foods ·* (Holland *et al,* 1991), with supplementary analysis data provided by manufacturers and added to the data base as previously described in chapter **2.** Phenylalanine analysis of potatoes was based on data from the Leatherhead Food Research Association/Laboratory of the Government

Chemist in London (Weetch *et al,* 1997). Energy intake was calculated as a percentage of the estimated average requirement for energy (DH, 1991).

3.3 Statistical analyses

Statistical analyses were by paired *t* tests and by a general linear model analysis of variance to compare differences using potato exchanges derived either from protein content or from direct phenylalanine content.

3.4 Results

There was no significant difference between pre-breakfast and pre-evening meal plasma phenylalanine concentrations when 50 mg phenylalanine exchanges for potato extrapolated from either protein or phenylalanine content were used.

3.4.1 Dietary intake

Subjects consumed a median extra 27 mg phenylalanine (an increase of 10%) and 1.0g natural protein (an increase of 18%) daily (Fig 3.2), when the new potato exchanges based on direct phenylalanine measurement were eaten. In addition, dietary energy ($p < 0.005$) (Fig 3.3), fat ($p < 0.05$) (Fig 3.4) and carbohydrate ($p < 0.001$) (Fig 3.5) all significantly increased, which was entirely due to the new potato exchanges which were boiled, mashed or fried (Table 3.2). Subjects consumed a median intake of 14 (range 7–29) potato exchanges per week in both weeks 1–3 and weeks 4–8.

Table 3.2

Energy, protein, fat and carbohydrate intake from natural foods

Nutrient	Mean intake		p
	Weeks 1 – 3	Weeks 4 – 8	
Energy kcal	1170	1238	<0.005
(SD)	(411)	(422)	
Protein g	11.3	13	NS
(SD)	(5.5)	(7)	
Fat g	44.2	46.4	<0.05
(SD)	(21.9)	(21.5)	
Carbohydrate g	186	198	<0.001
(SD)	(72)	(78)	
% Energy of EAR	96	101	<0.0001
(SD)	(19.8)	(18.9)	
% Protein of RNI	231	236	<0.0001
(SD)	(60)	(61)	
% kcal protein	15%	15%	NS
% kcal fat	23%	25%	NS
% kcal carbohydrate	58%	58%	NS

3.4.2 Plasma phenylalanine concentrations

There was no significant difference between mean pre-breakfast plasma phenylalanine concentrations between weeks 1–3 (mean 336 μmol/l: SD 213) and weeks 4–8 (mean 322 μmol/l: SD 211) (Fig 3.6). Equally, there was no difference between pre-evening meal plasma phenylalanine concentrations between the same two study periods (mean plasma phenylalanine:- weeks 1–3: mean 309 μmol/l; SD 234; weeks 4–8: mean 310 μmol/l, SD 233) (Fig 3.7). The median (range) change in pre-breakfast and pre-evening meal plasma phenylalanine concentration between weeks 1–3 and weeks 6 – 8 was -4 μmol/l (-227 to 100 μmol/l) and 36 μmol/l (-283 to 134 μmol/l) respectively (Fig 3.8).

3.5 Summary

This study provides clear evidence that the new phenylalanine content can be safely used for potato exchanges in PKU. This is despite a median increase in phenylalanine intake of 27 mg daily. Previous analysis, extrapolated from the protein content of potatoes, overestimated the phenylalanine content, thus reducing the portion size for these staple foods in PKU. Thus, the new phenylalanine analysis data for potatoes has allowed relaxation in this difficult and rigid diet.

Fig 3.2

Daily extra phenylalanine and protein consumed by using potato exchanges based on direct phenylalanine measurement
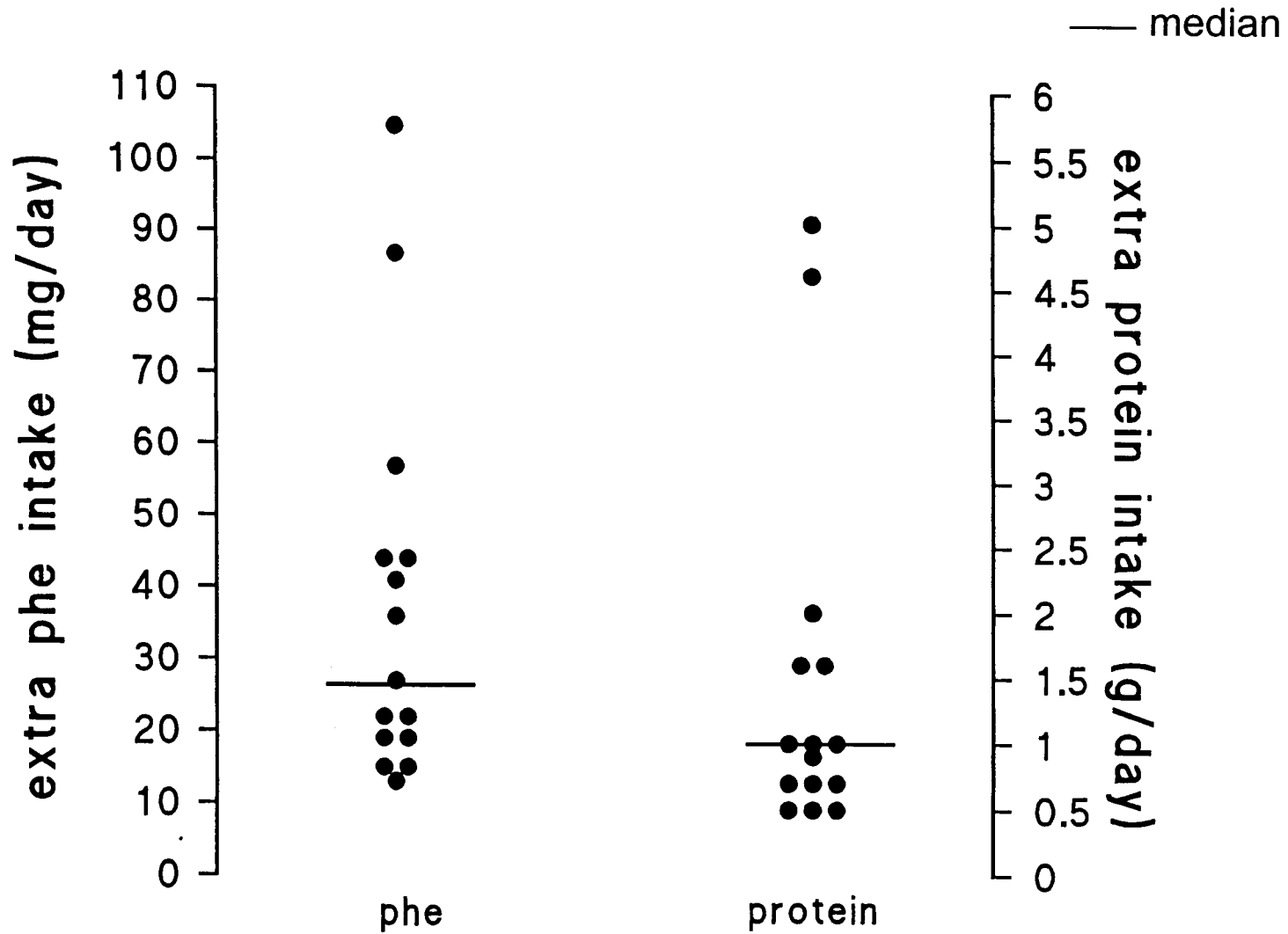
Fig 3.3   Effect on energy intake of potato exchanges with phenylalanine content calculated from protein concentration and those based on direct phenylalanine measurement

— mean
p<0.005
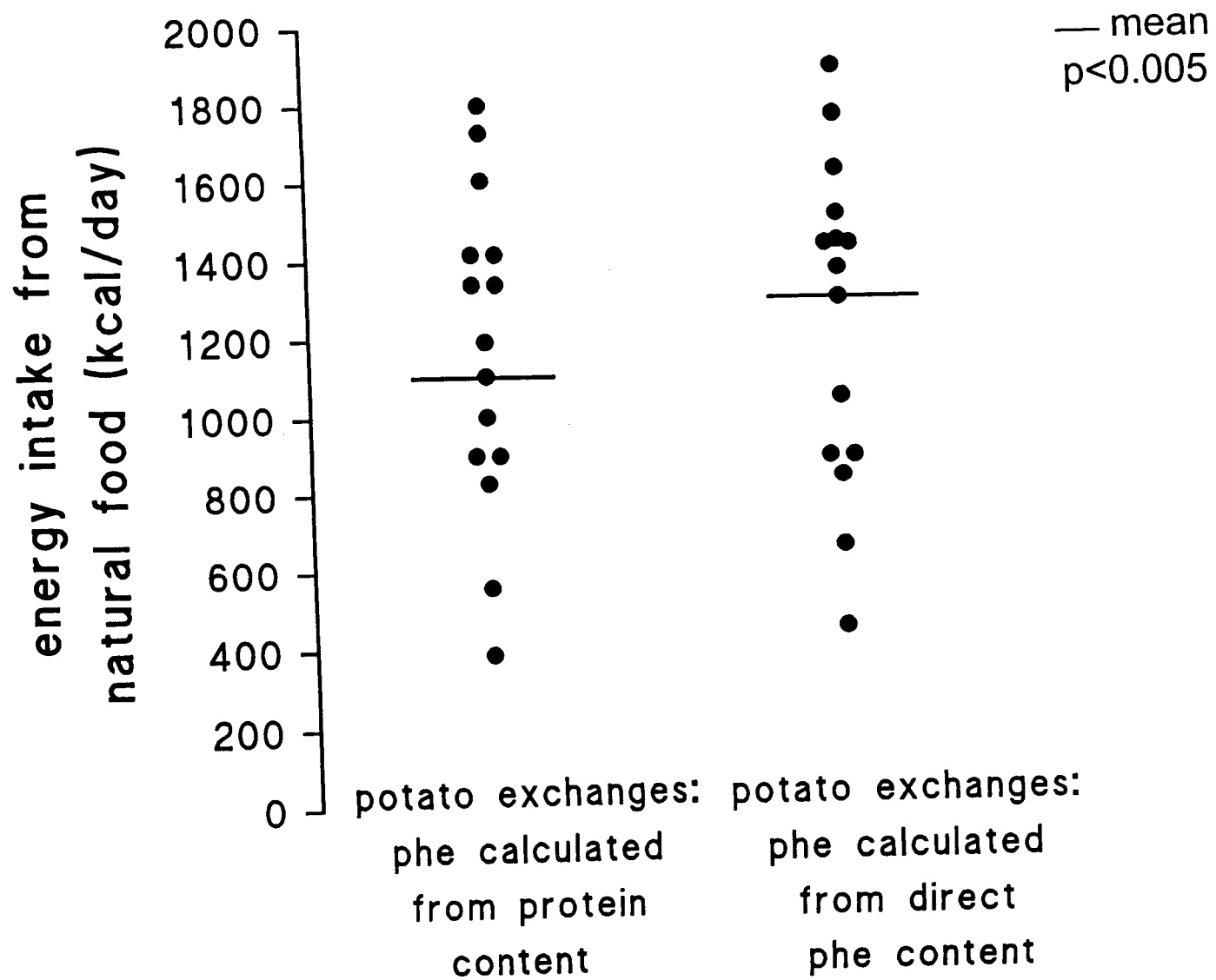
Fig 3.4　　**Effect on fat intake of potato exchanges with phenylalanine content calculated from protein concentration and those based on direct phenylalanine measurement**

— mean
p<0.005

fat intake from natural food (g/day)

potato exchanges: phe calculated from protein content

potato exchanges: phe calculated from direct phe content
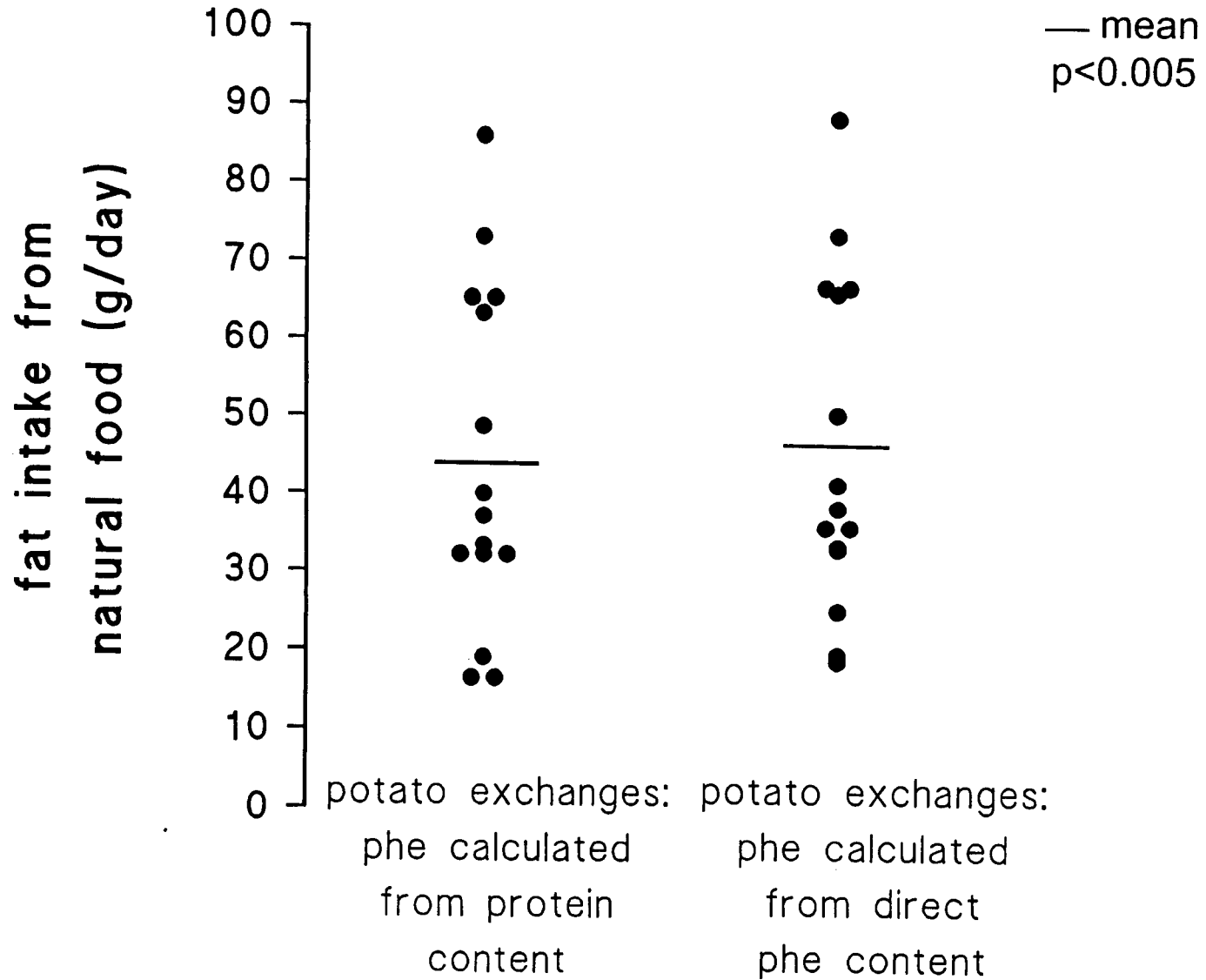
Fig 3.5   Effect on carbohydrate intake of potato exchanges with phenylalanine content calculated from protein concentration and those based on direct phenylalanine measurement
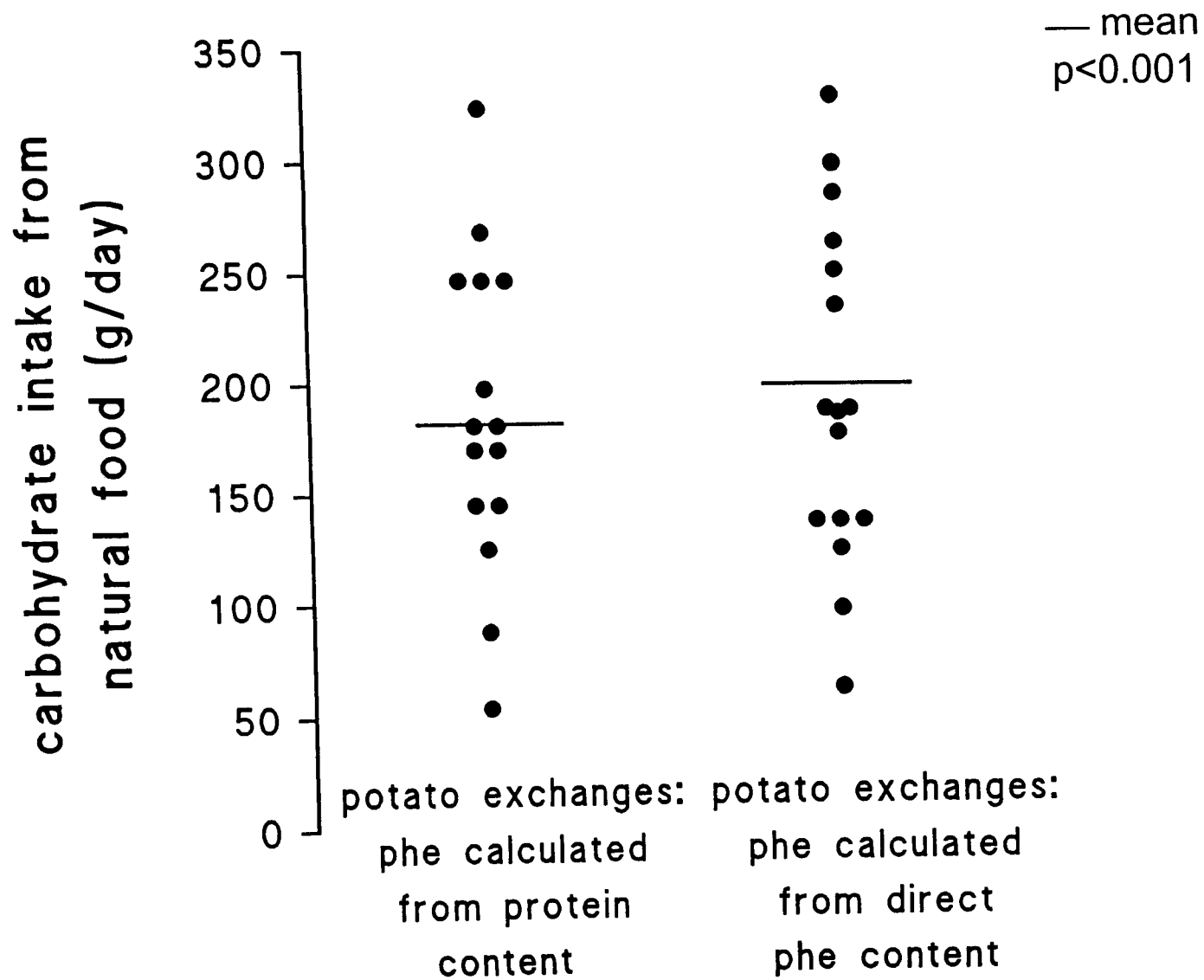
Fig 3.6   Effect on morning plasma phenylalanine concentrations
of potato exchanges calculated from protein concentration and
those based on direct phenylalanine measurement
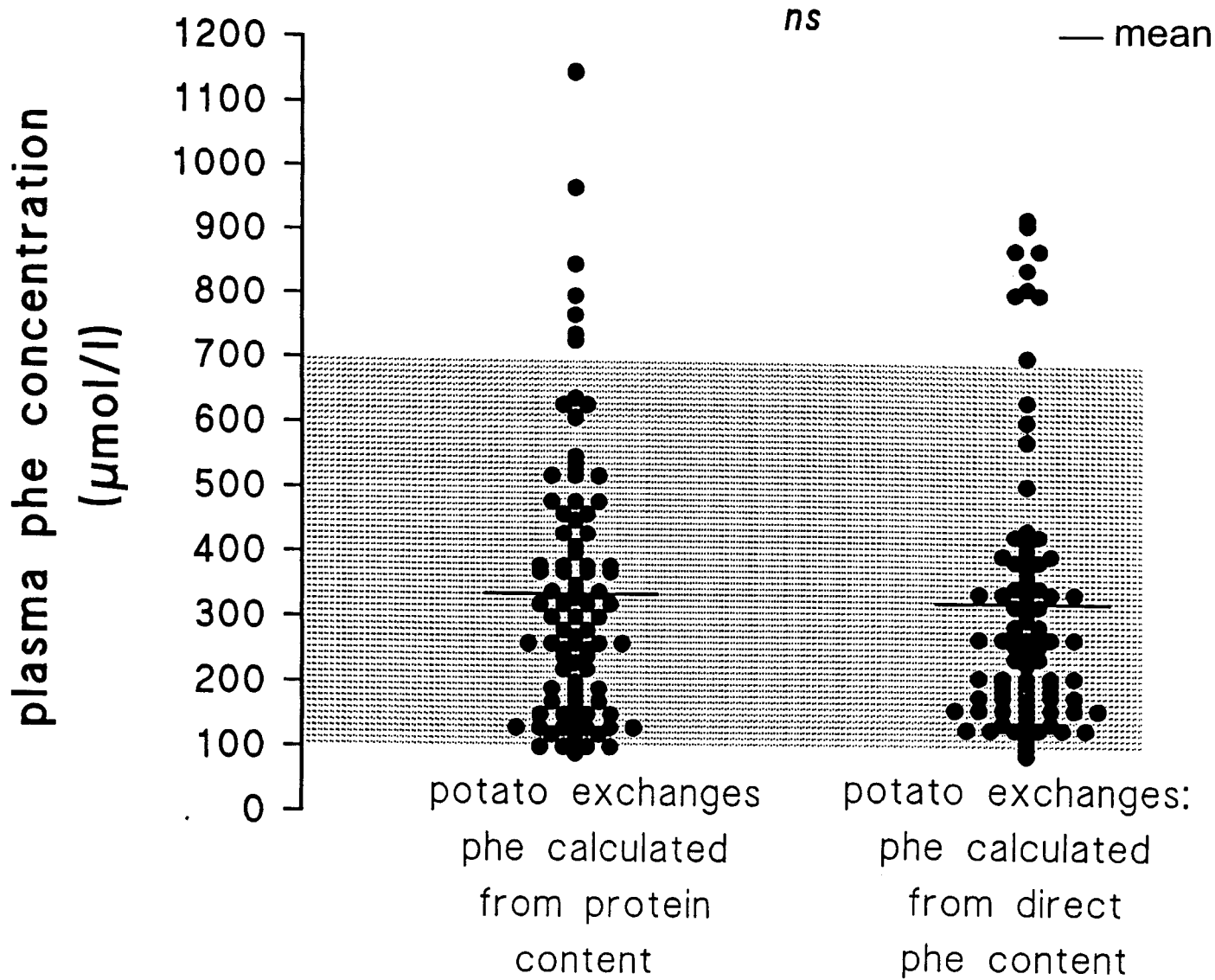
Fig 3.7   Effect on evening plasma phenylalanine concentrations of potato exchanges calculated from protein concentration and those based on direct phenylalanine measurement
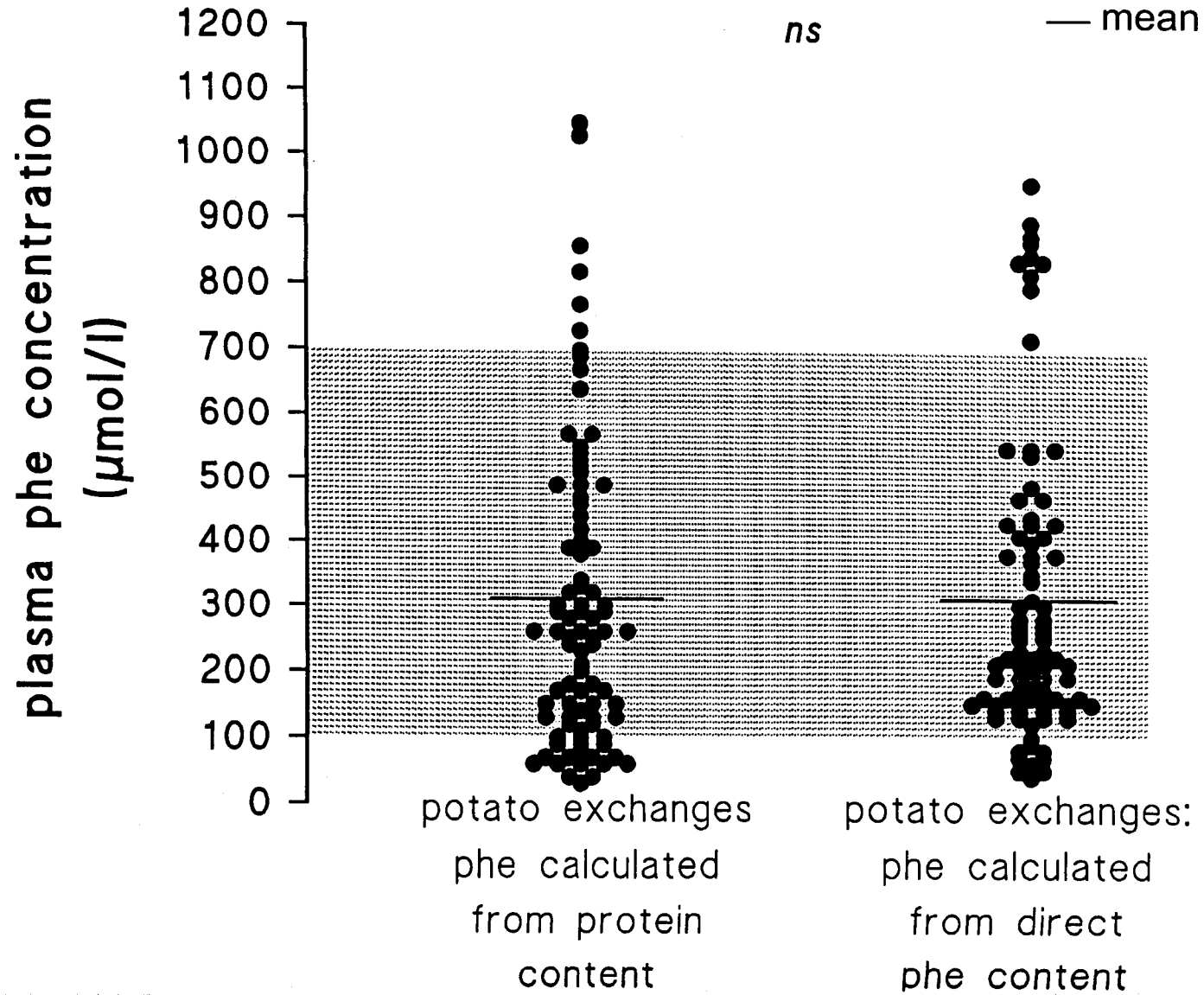
Fig 3.8   Change between weeks 1 - 3 (potato exchanges calculated from protein content) and weeks 6-8 (potato exchanges based on direct phenylalanine measurement) morning and evening plasma phenylalanine concentrations
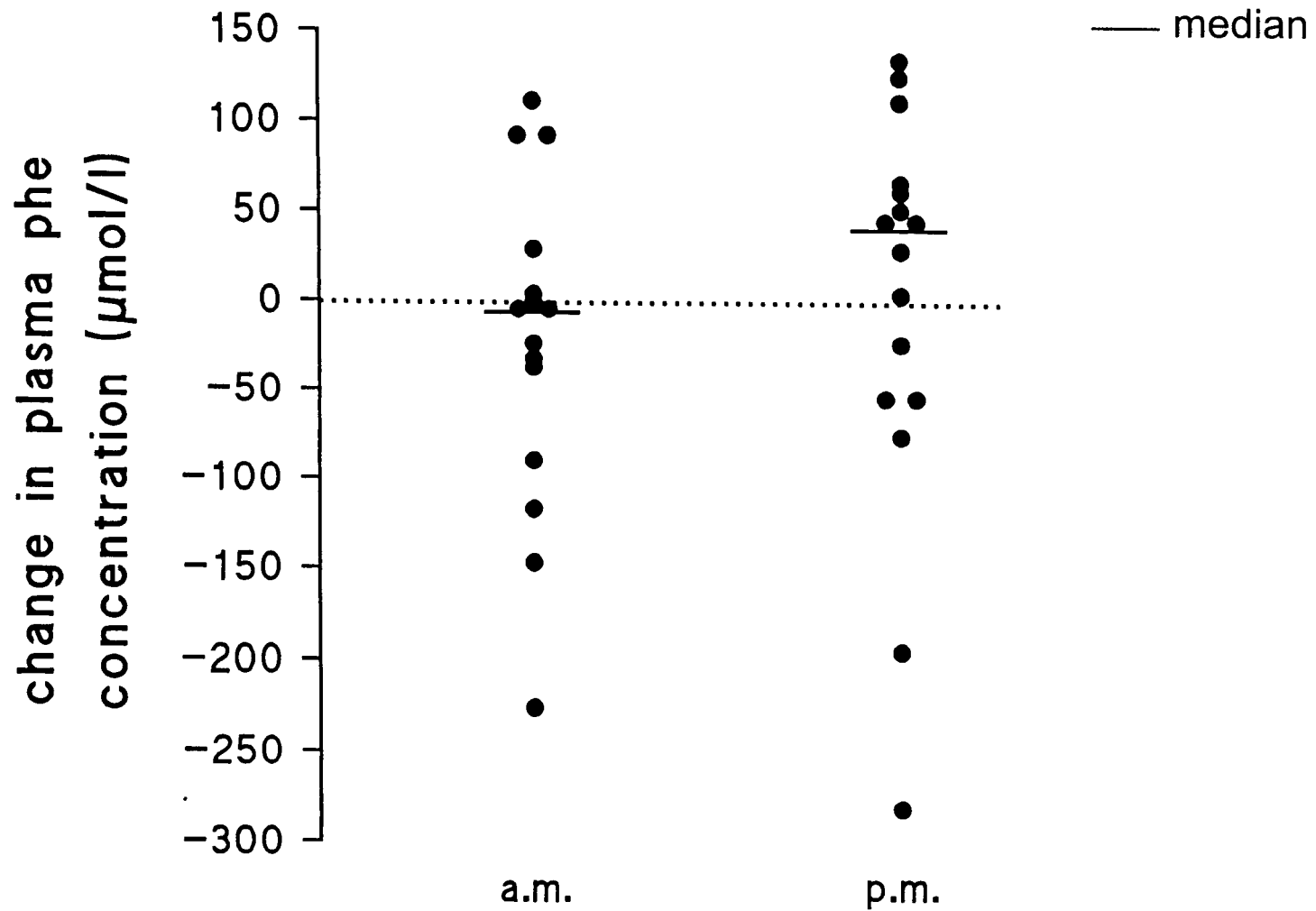
Chapter 4

A prospective, cross-over trial investigating the effect of free use of fruit and vegetables containing intermediate amounts (51-100 mg/100g) of phenylalanine on control in PKU

4.1 Introduction

There is no rational approach to the allocation of fruit and vegetables containing phenylalanine between 51–100 mg/100g in the PKU diet. Some are incorporated as part of the 50 mg phenylalanine exchange system; others are freely allowed in the diet without measurement.

In chapter 2, the study examining factors affecting variation in plasma phenylalanine concentrations demonstrated that natural protein **was** increased by almost 50% from mainly 'free' foods, such as fruit and vegetables but this did not destabilise overall control. That study included some of the permitted vegetables containing intermediate amounts of phenylalanine (51–100 mg/100g) freely, but others were counted as exchanges. It is possible that all fruit and vegetables containing 51-100 mg/100g of phenylalanine could be permitted freely in the PKU diet. However, their free use has not been investigated. The aim of this study was to systematically evaluate the effect of the free use of fruit and vegetables containing phenylalanine between 51–100 mg/100g on patients with PKU.

4.2 Subjects and methods

4.2.1 Subjects

Seventeen subjects (*16* Caucasian, 1 Afio-Caribbean) were recruited into the study. There were 15 girls and 2 boys with a median age of *6* years (range 1–24 years). All had classical PKU. There were four inclusion criteria: 1) maintenance of at least 70% plasma phenylalanine concentrations (weekly, fortnightly or monthly according to age group) within ranges recommended by the MRC Working Group (1993) in the *6* months before entering the study;

2) age over 1 year; 3) ability to eat fruit and vegetables containing phenylalanine either between 50–75 mg/100g or 76–100 mg/100g; 4) demonstrable parental ability to take skin puncture blood specimens.

Part 1 and 2: Fruit and vegetables containing phenylalanine between 51–75 mg/100g

Fifteen subjects (13 girls; 2 boys) entered this part of the study. Their median age was 6 years (range 2–24 years), and their median daily intake of 50 mg phenylalanine exchanges was 6 (range 5–16). Three patients did not continue to complete part 3 of the study, as they disliked the vegetables in this category.

Part I, 2, and 3: Fruit and vegetables containing phenylalanine between 76-100mg/100g

Fourteen subjects (12 girls and 2 boys) entered this part of the study. Their median age was 6 years (range 1–19 years) and their median daily intake of 50 mg phenylalanine exchanges was 6 (range 5–16). Two subjects omitted weeks 4–8 of part 2 of the study as they disliked these fruit and vegetables but continued part 3.

All patients were eating 50 mg phenylalanine potato exchanges based on phenylalanine content rather than calculated from protein analysis. The following amounts of total protein from protein substitute and phenylalanine exchanges were allocated:

age <2 years: 3.0g/kg/day; 2–5 years: 2.5g/kg/day; 6–10 years: 2.0g/kg./day; over 11 years of age: 1.5g/kg/day. Patients were given their usual brand of protein substitute. This was taken

either as a paste or drink. Ten patients used *XP* Maxamaid (Scientific Hospital Supplies), and five used Phlexy 10Drink Mix (Scientific Hospital Supplies), one used PKU Aid **4** (Scientific Hospital Supplies), and one used XP Maxamum (Scientific Hospital Supplies).

This study was approved by the Committee on Medical Ethics of South Birmingham Health Authority. Informed consent was obtained from all parents and all competent patients.

4.2.2 Study design

This was a three-part, open, prospective, cross-over study conducted at home. The free use of fruits and vegetables containing phenylalanine between 0–50 mg/100g, 51-75 mg/100g and 76-100mg/100g mg was studied sequentially. Patients could take part in two or all three parts of the study. This was determined by their ability to eat the different fruit and vegetables under study.

Part 1: Fruit and vegetables containing phenylalanine 0–50 mg/100g

During weeks 1–3, subjects ate only fruit and vegetables containing phenylalanine **<50** mg/100g freely. Twice daily serial skin puncture blood specimens for plasma phenylalanine were collected over the last **3** days on weeks 1 and 3. Specimens were always collected pre-breakfast and pre-evening meal. They followed the same standard menu on blood sampling days in week 3 **as** eaten in week 1.

Part 2: Fruit and vegetables containing phenylalanine 51–75 mg/100g

During the weeks **4-8,** subjects ate at least one portion daily of fruits and vegetables containing

phenylalanine 51-75mg/100g as free food. This included foods such as bananas, mushrooms

and raisins (Table 4.1).

Table 4.1

Protein and phenylalanine content *of* fruit and vegetables containing phenylalanine between 51-100 mg/100g

Fruit and vegetables	Phenylalanine mg/100g	Protein g/100g	Current Status
Phenylalanine 51-75mg/100g			
Raisins	51	2.1	free
Banana	53	1.2	100g/exchange
Leeks	53	0.7	free
Sultanas	58	2.7	free
Mushrooms	65	1.8	free
Mange tout	66	3.2	30g/exchange
Avocado	71	1.9	55g/exchange
Mushrooms, fried	75	2.4	free
Phenylalanine 76-100mg/100g			
Broccoli	76	3.1	30g/exchange
Asparagus	**84**	3.4	1 servingiday
Cauliflower	89	2.9	
Brussels sprouts	92	2.9	35g/exchange
Beansprouts	93	2.9	1 serving day
Yam	97	1.7	60g/exchange

A portion was defined **as** that containing at least 20g of fruit and vegetable. Twice-daily serial skin puncture blood specimens were always collected pre-breakfast and pre-evening meal over the last 3 days on weeks *6* and 8. They also followed the same standard menu on blood sampling days **as** week 1, but during weeks **6** and **8** at least one portion of fruit and vegetable containing phenylalanine between *51-75* mg/100g was added to the menu.

Part 1,2, and 3: Fruit and vegetables containing phenylalanine 76-100 mg/100g.

Continuing from the first two parts of the study, subjects consumed **3** portions per week of vegetables containing phenylalanine between 76–100 mg/100g for a further 7 weeks (Table **4.1).**
A portion was defined **as** that containing at least 20g of vegetable. Over the last **3** days of weeks 11, 13 and 15, additional twice daily serial skin puncture blood specimens were taken pre-breakfast and pre-evening meal. They followed the same standard menu as for week 1, but also, when possible, continued to eat fruits and vegetables containing phenylalanine between 51-75 mg/100g on blood sampling days. They also consumed at least one portion daily of vegetables containing phenylalanine between **76-100** mg/100g on blood sampling days.

Assessment of plasma phenylalanine concentrations

Parents or subjects took heel and thumb skin puncture blood specimens at home, and immediately posted them to the hospital. Blood collection technique had been taught and competence assessed by a specialist nurse. All blood specimens were centrifuged upon receipt

and resulting plasma samples stored at -20°C until analysed. Plasma phenylalanine concentrations were measured by HPLC as previously described.

All patients were free of intercurrent infections on the days of the blood sampling. If a subject became ill immediately before or during blood sampling days, the plasma phenylalanine measurements and standard menu were deferred until the patient had recovered.

Assessment of dietary intake

Throughout the study, parents or subjects kept a diary of any fruit and vegetables eaten containing phenylalanine between 51-75 mg/100g or 76–100 mg/100g. On blood sampling days, the only food and drink parents or carers were instructed they could vary were very low phenylalanine foods (containing <0.3g/100g of protein) such as sweets and squash drinks. They were also instructed to keep to the same times for administration of protein substitute on these days. All food and drink was weighed using Salter scales (accurate to 5g), about which parents or older subjects had been instructed. Only food and drink actually consumed was recorded; any remaining food was deducted from the total start weight.

Nutritional analysis was calculated using the Microdiet computer programme based on *McCance* & *Widdowson's* *"The Composition of Foods* " (Holland *et al,* 1991), with supplementary analysis data provided by manufacturers and added to the data base as previously described in chapter **2.** Phenylalanine analysis of fruit and vegetables was based on data from the Leatherhead Food Research Association and Laboratory of the Government Chemist in

London (Weetch, 1997). Energy intake was calculated as a percentage of the estimated average requirement for energy (DH, 1991).

4.3 Statistical analyses

Statistical analyses was by paired *t* tests to compare differences between weeks 1–3 and 4–8, and weeks 1–3 and 9–l 5. Repeated Measures Analysis of Variance, and Tukey-Kramer Multiple Comparison Test were used to compare differences between each part of the fruit and vegetable study, i.e. weeks 1-3, 4-8 and 9–15.

4.4 Results

4.4.1 Part 1 and 2: Addition of fruit and vegetables containing phenylalanine 51-75mg/100g

The free use of fruit and vegetables containing phenylalanine between 51-75 mg/100g did not adversely affect plasma phenylalanine control. There was no significant difference in pre-breakfast plasma phenylalanine concentrations between weeks 1–3 (mean: 318 μmol/l; SD 175) when only fruit and vegetables containing phenylalanine <50 mg/100g were given and weeks 4–8 (mean: 312 μmol/l; SD 196), when at least one portion of fruit and vegetable containing phenylalanine between 51-75mg/100g was given daily (Fig 4.1). Nor was there any difference in pre-evening meal plasma phenylalanine concentrations between the study periods (mean plasma phenylalanine:- weeks 1–3: 273 μmol/l SD 184; and weeks 4–8: 282 μmol/l; SD 222) (Fig 4.2).

Subjects ate an extra medium of 51 mg (range 30–138mg) of phenylalanine daily from fruit and

vegetables containing phenylalanine between 51-75 mg/100g on the days of dietary assessment

(Fig 4.3). This increased phenylalanine intake by a median of 15% (range 5–55%) over

allocated exchanges (Fig 4.3). *An* additional median of 1.3g protein (range 0.7-3.2g) daily

came from this source and excess protein over allocated exchanges from all free foods increased

from a mean of 46% (SD 27) in weeks 1–3 to 68% (SD 30) in weeks 4–8 (p <0.0001) (Fig 4.4).

Natural protein (p <0.005) (Fig 4.9, energy (p <0.001), (Fig 4.6) and carbohydrate (p <0.005)

(Fig 4.7), but not fat intake significantly increased between weeks 1–3 and 4–8 (Table 4.2).

The mean % **EAR** for energy (weeks 1–3: 94.8%, SD 16.3; weeks 6-8: 99.9%, **SD** 17.7;

p <0.001) and the RNI for protein (weeks 1–3: 256%, SD 56; weeks **6–8: 265%,** SD 61;

p <0.0001) also significantly increased with the addition of fruit and vegetables containing

phenylalanine between 51–75 mg/100g. This was entirely due to the protein and carbohydrate

in the fruit and vegetables sources. The percentage energy provided by protein (p <0.05) and fat

(p *<0.05)* decreased slightly but this was significant; the percent energy provided by

carbohydrate increased but this was not quite significant (p <0.09). The median daily weight of

fruit and vegetables containing phenylalanine between 51–75 mg/100g consumed was 83g

between weeks 4–8.

4.4.2 Part 1, 2 and 3: Addition of fruit and vegetables containing phenylalanine 76-100mg/100g

The free addition of vegetables containing phenylalanine between 76–100 mg/100g freely in the

diet did not destabilise blood phenylalanine control. There was no significant difference in pre-

Table 4.2

Nutrient intake during the study

Nutrient	Mean Intake		
	Part 1 **Weeks 1 – 3**	**Part 2** **Weeks 4 – 8**	**Part 3** **Weeks 9 – 15**
	excluding protein substitute	excluding protein substitute	excluding protein substitute
Energy kcal (SD)	1014 (SD 282)	1089 (SD 317)	1085 (SD 331)
Protein g (SD)	10.4 (6.3)	11.7 (6.3)	13.2 (6.9)
Fat g (SD)	33.2 (10.7)	32.2 (11.2)	33.3 (11.8)
Carbohydrate g (SD)	172 (53)	190 (59)	190 (66)
	including protein substitute	including protein substitute	including protein substitute
% kcal/protein	15.8 (3.2)	15.6 (3.3)	15.9 (3.9)
% kcal/fat (SD)	20.7 (5.8)	20 (5.7)	19.7 (5.8)
% kcal/carbohydrate	61.4 (5.4)	62.2 (6.1)	62.7 (5.1)
% kcal/EAR (SD)	94.8 (16.3)	99.9 (17.7)	97 (16.3)
% Protein/RNI (SD)	256 (56)	265 (61)	257 (46)

breakfast plasma phenylalanine concentrations between weeks 1–3 when no fruit and vegetables

containing phenylalanine between **51-100** mg/100g were eaten (mean plasma phenylalanine:

326 µmol/l, SD 183); and in weeks 9–15 when three portions were eaten each week (mean

plasma phenylalanine 272 µmol/l, SD 199) (Fig 4.8). Nor was there any difference in pre-

evening meal plasma phenylalanine concentrations between the study periods (mean plasma

phenylalanine:- weeks 1–3: 274 µmol/l (SD 193); weeks 4–8: 273 µmol/l (SD 208);

weeks 9–15: 270 µmol/l (SD 204)) (Fig 4.9).

The vegetables containing phenylalanine between **76-100** mg/100g increased phenylalanine

intake by a median of 39 mg daily (range 13–143 mg) (Fig 4.10). Overall, the protein intake

provided by all 'free' foods in excess of allocated exchanges increased from 46% between

weeks 1–3 to 64% between weeks 4–8 and 86% between weeks 9–15 (weeks 1–3 vs

rest p <0.001) (Fig 4.11). Although total protein (p <0.005) significantly increased,

carbohydrate only slightly improved and fat intake remained unchanged (Table 4.2) between

weeks 1–15. There was no significant change in the distribution of energy between

carbohydrate, fat and protein. The median portion size of vegetables containing phenylalanine

between 76-100mg consumed was 50g (10-150g) at least 3 times weekly from weeks *9-15.*

4.5 Summary

This study demonstrates that fruit and vegetables containing between **51-100** mg of

phenylalanine did not adversely affect control when permitted without restriction in PKU. They

provided a substantive source of dietary phenylalanine; increasing daily phenylalanine by a

median of 51 mg and 39 mg respectively in the two test periods. Their lack of apparent affect on blood phenylalanine concentrations is surprising. However, satisfactory portion sizes were tested over prolonged time periods. This data, therefore, provides an opportunity to examine and redefine the allocation of these fruits and vegetables in the diet.

Fig 4.1 Comparison of fruit and vegetables containing phenylalanine <50mg/100g and 51-75mg/100g on morning plasma phenylalanine concentrations
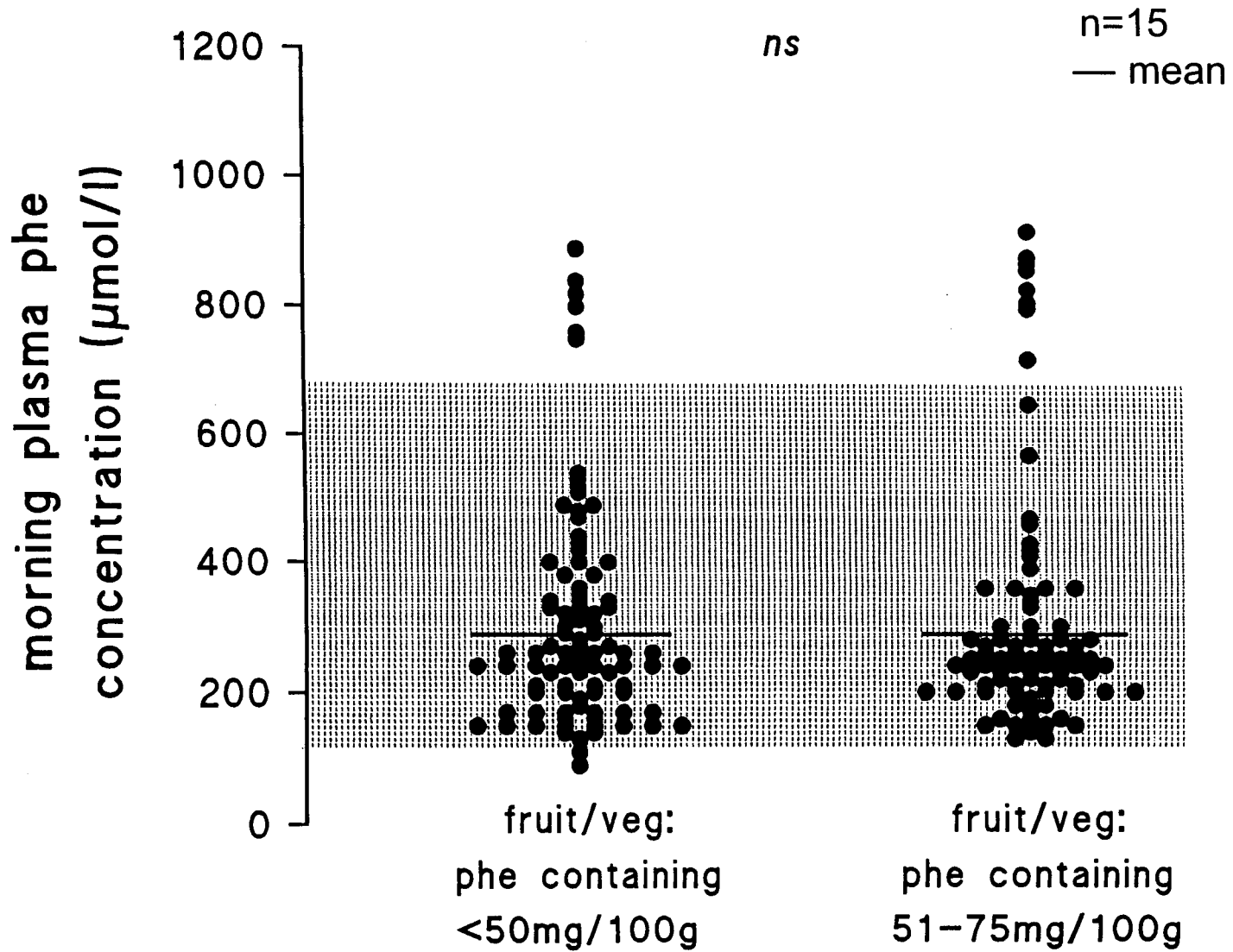
Fig 4.2 **Comparison of fruit and vegetables containing phenylalanine <50mg/100g and 51-75mg/100g on evening plasma phenylalanine concentrations**
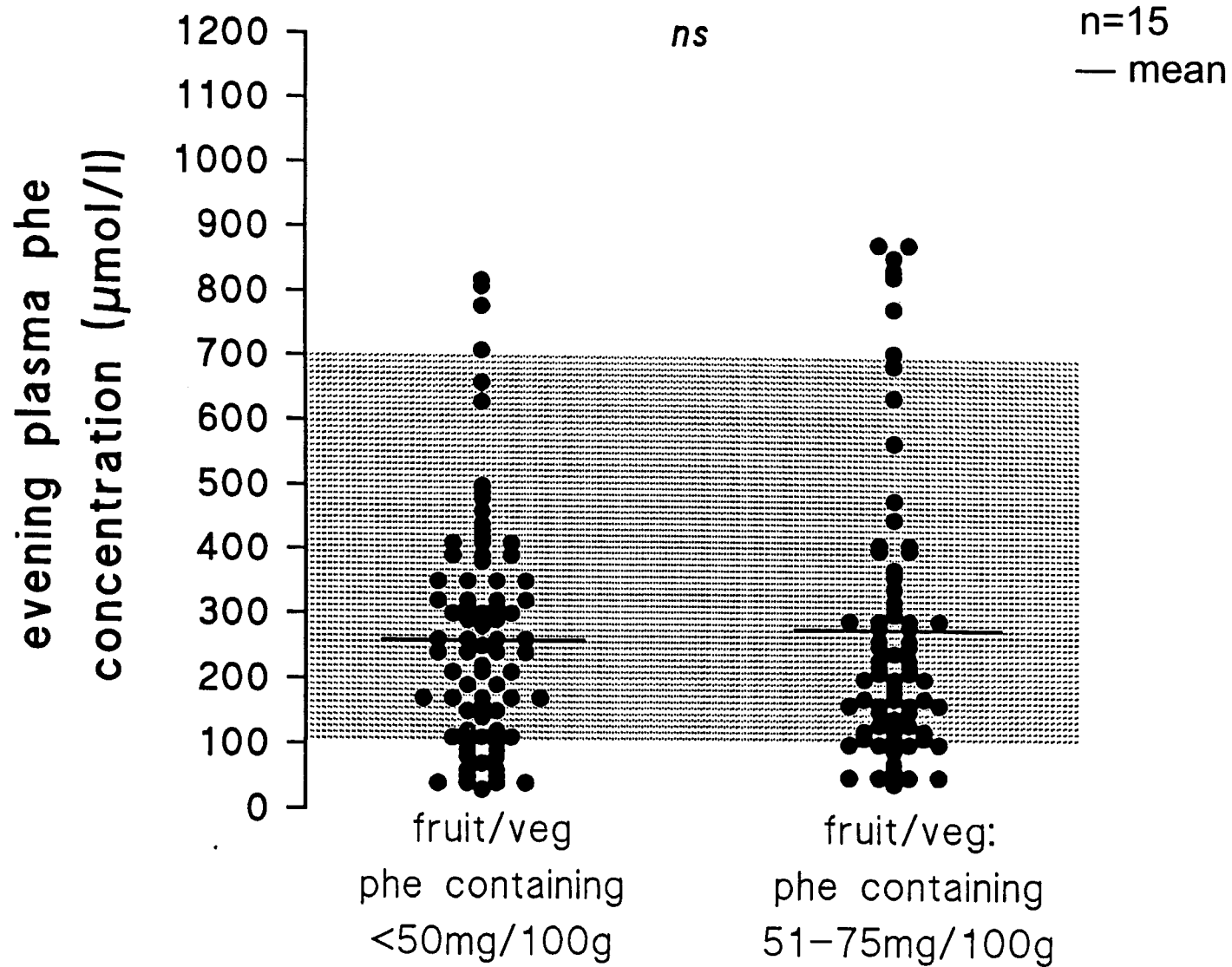
Fig 4.3   Additional dietary phenylalanine from the free use of fruit and vegetables containing phenylalanine 51-75mg/100g
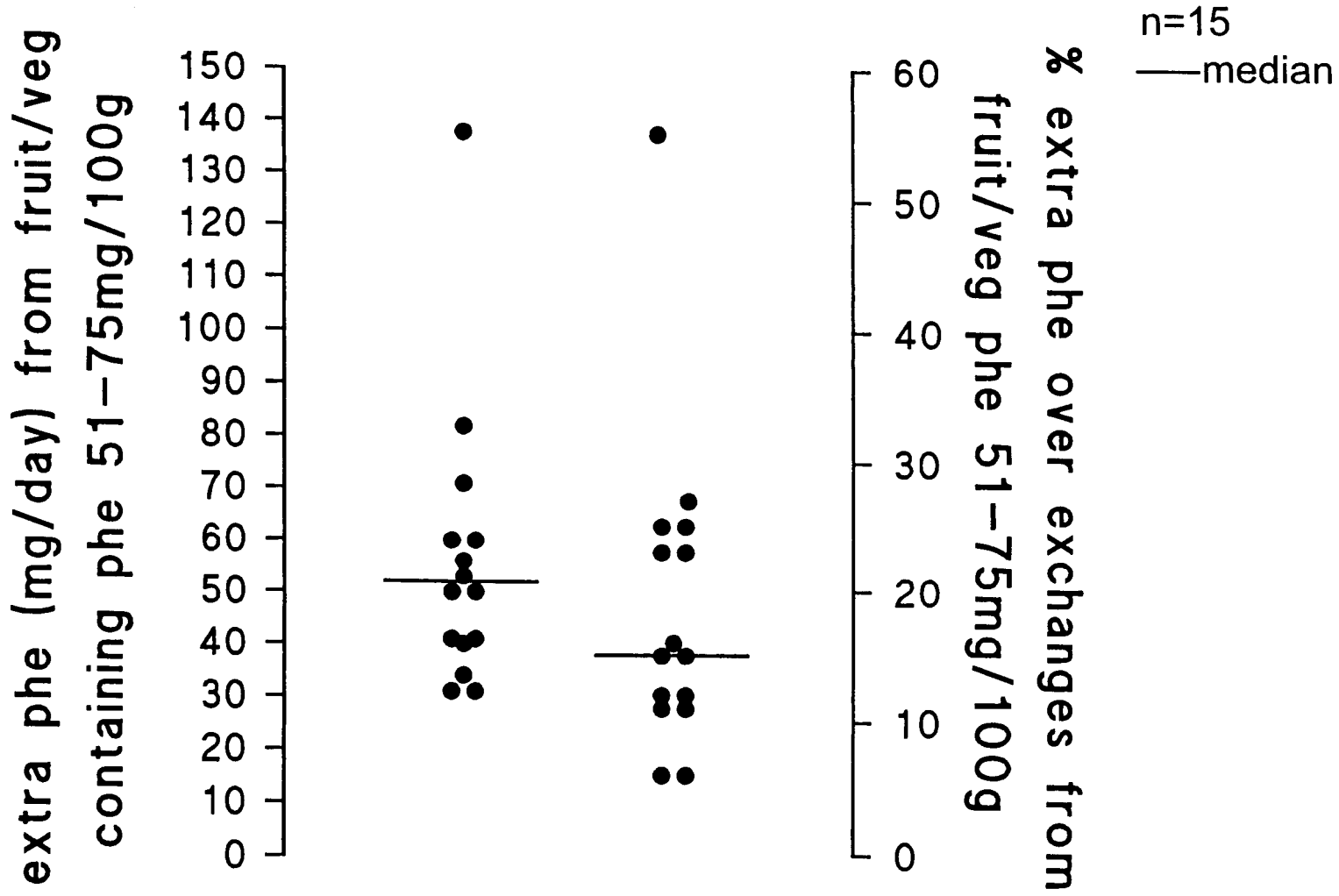
n=15
——median

Fig 4.4   Comparison of % excess natural protein from freely allowed foods with fruit and vegetables containing phenylalanine <50mg and 51-75mg/100g

n=15
— mean
p<0.0001

% extra protein over phe exchanges

fruit/veg:
phe containing
<50mg/100g

fruit/veg:
phe containing
51–75mg/100g
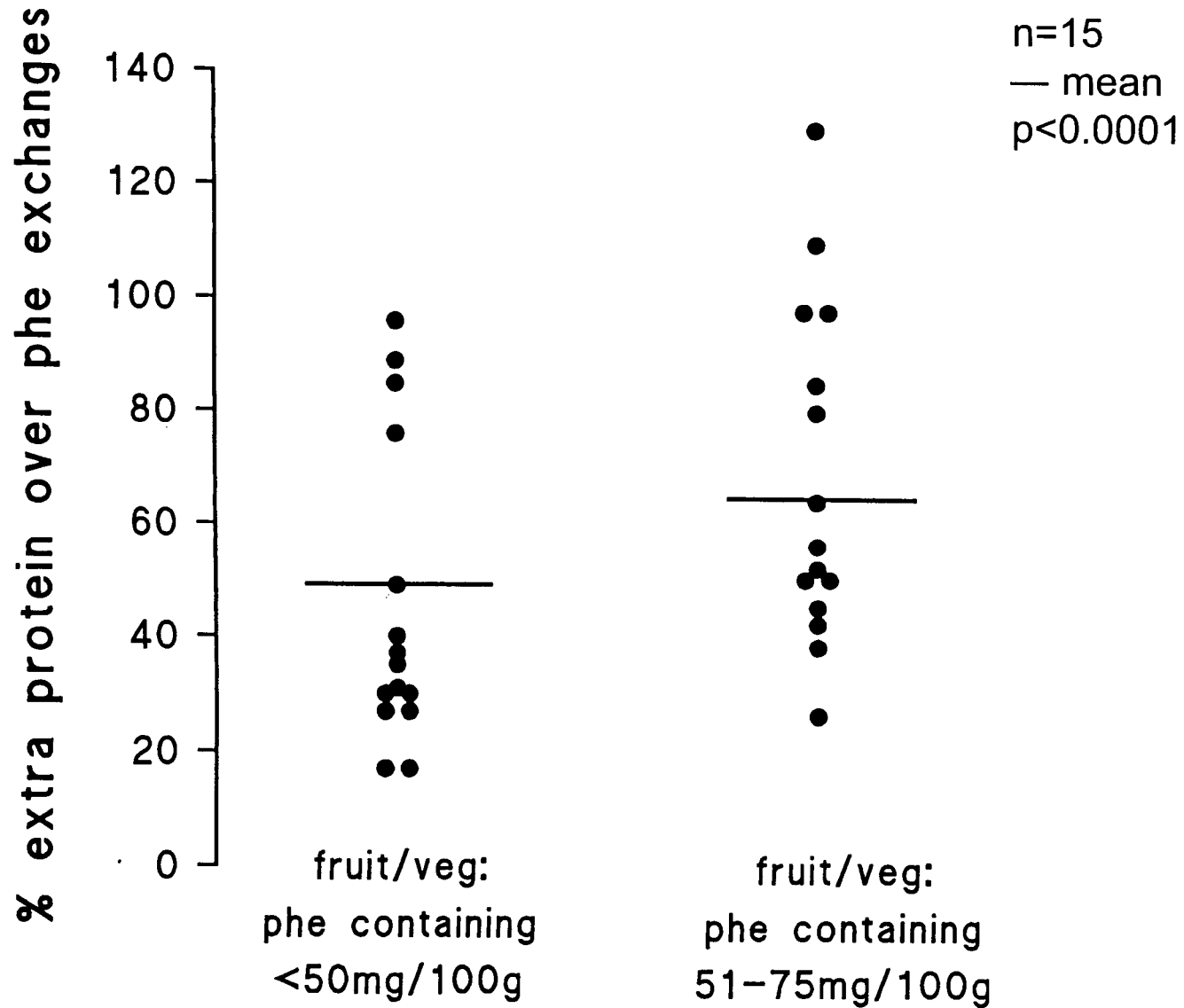
Fig 4.5 **Change in natural protein intake with the addition of fruit and vegetables containing phenylalanine 51-75mg/100g**

n=15
— mean
p<0.0005

Protein intake (g/day)

fruit/veg:
phe containing
<50mg/100g

fruit/veg:
phe containing
51−75mg/100g
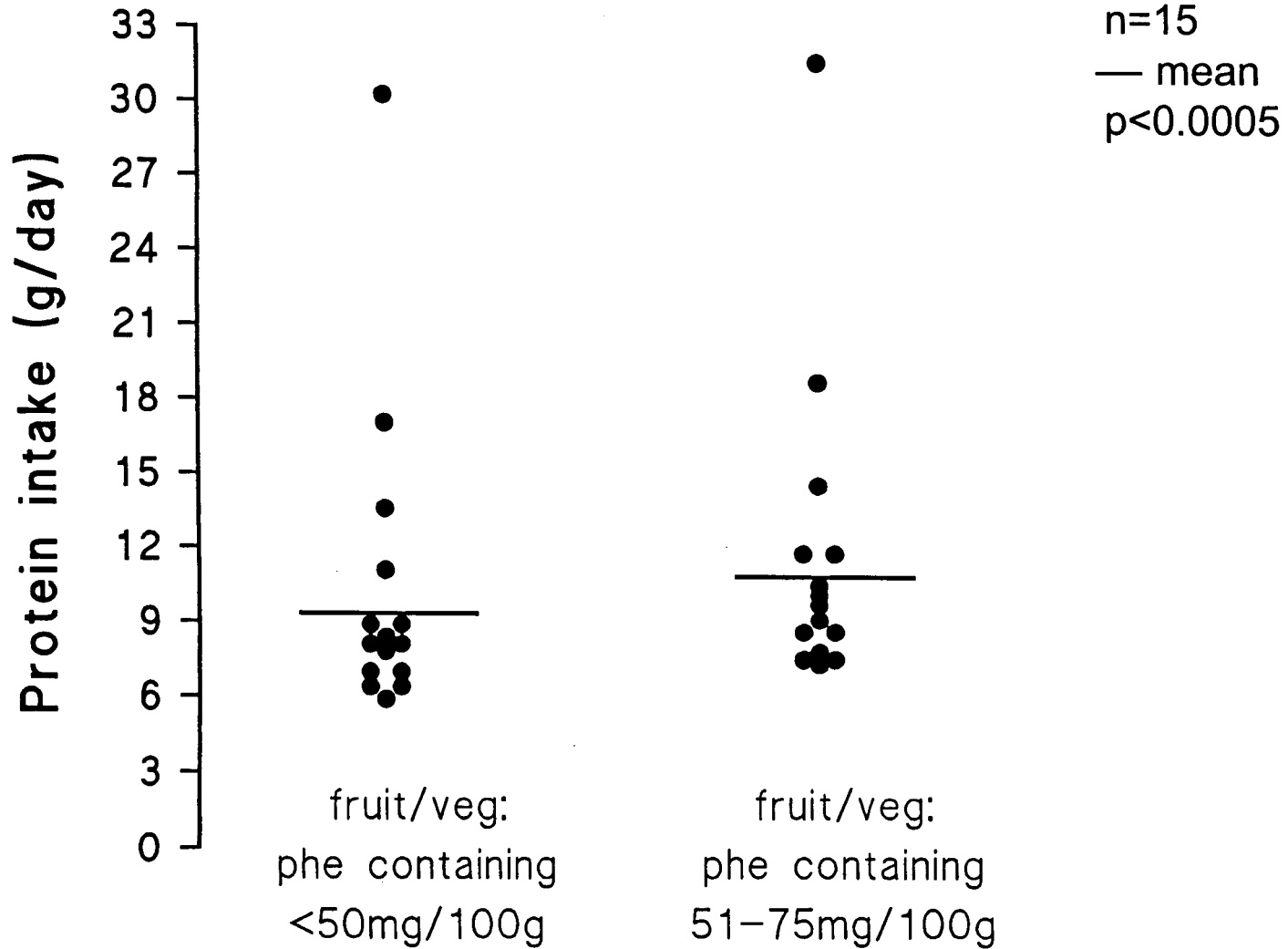
Fig 4.6   **Comparison of fruit and vegetables containing phenylalanine <50mg and 51-75mg/100g on energy intake**

n=15
— mean
p<0.001

energy intake (kcal/day)

fruit/veg:
phe containing
<50mg/100g

fruit/veg:
phe containing
51−75mg/100g
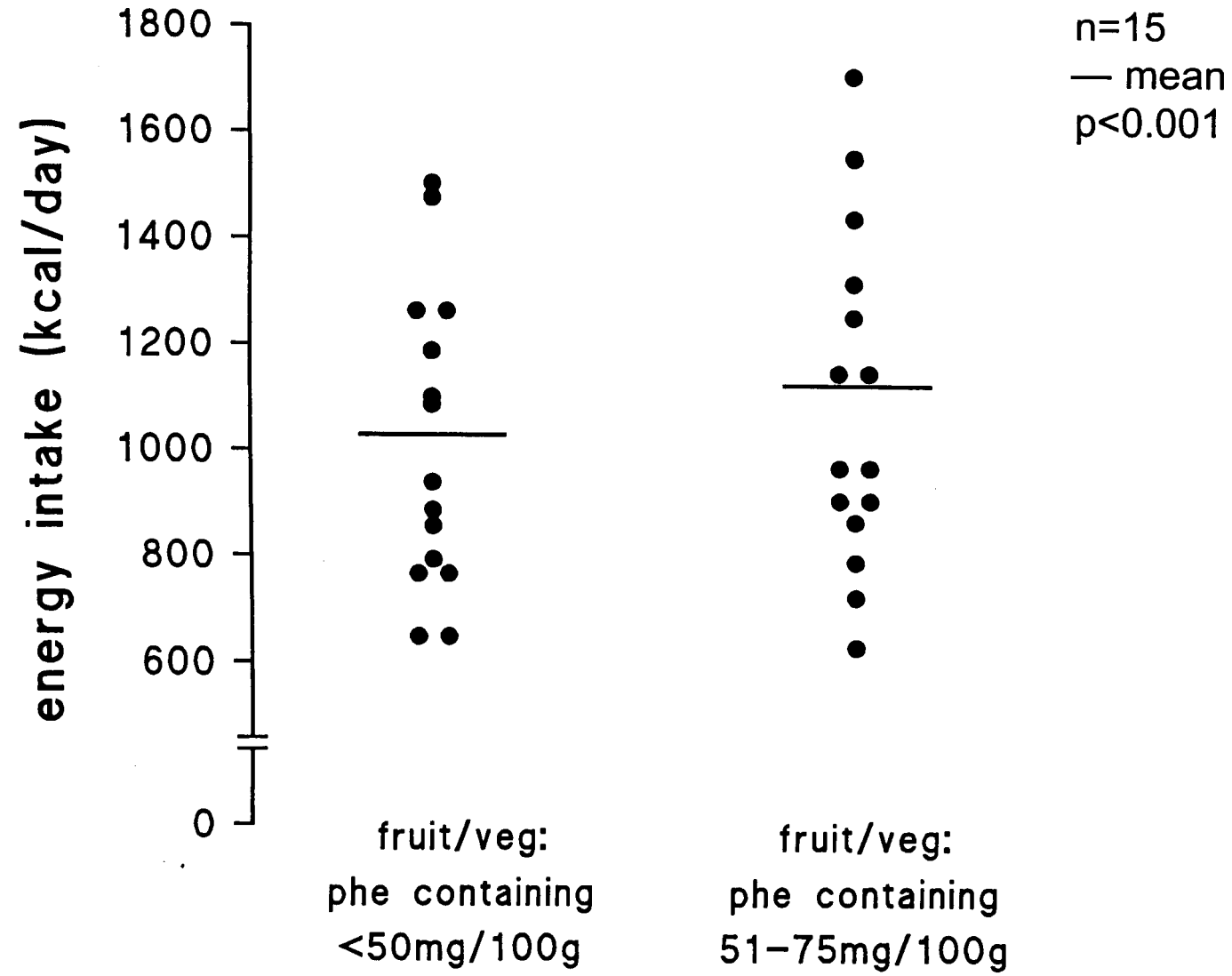
Fig 4.7   Comparison of carbohydrate intake from fruit and vegetables containing phenylalanine <50mg and 51-75mg/100g

n=15
— mean
p<0.005

carbohydrate intake (g/day)

fruit/veg:
phe containing
<50mg/100g

fruit/veg:
phe containing
51-75mg/100g
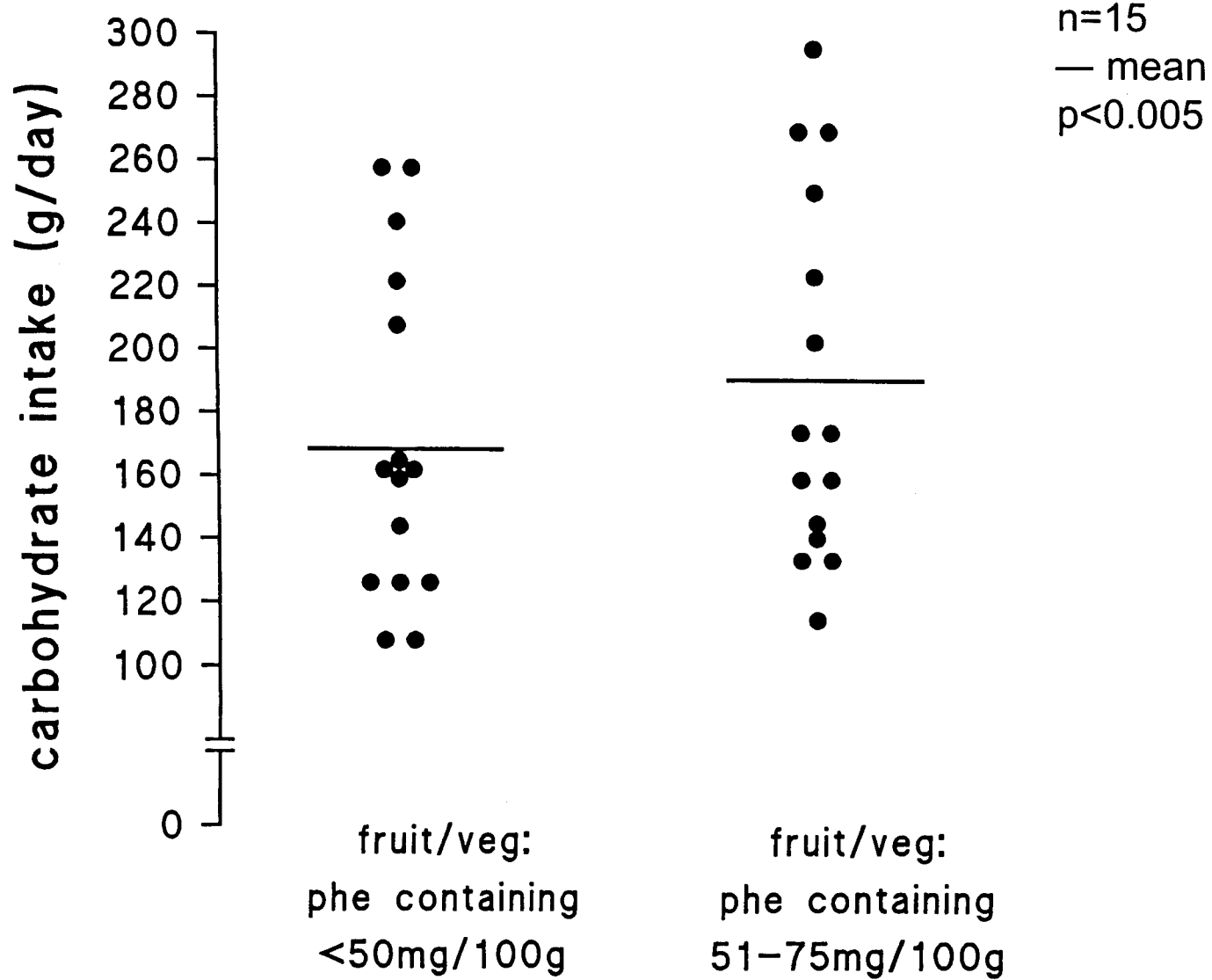
Fig 4.8

Comparison of free use of fruit and vegetables containing phenylalanine <50mg, 51-75mg, and 76-100mg/100g on morning plasma phenylalanine concentrations

n=14
— mean

ns

morning plasma phe concentration (µmol/l)

1200
1000
800
600
400
200
.0

fruit/veg:
phe containing
<50mg/100g

fruit/veg:
phe containing
51-75mg/100g

fruit/veg:
phe containing
76-100mg/100g
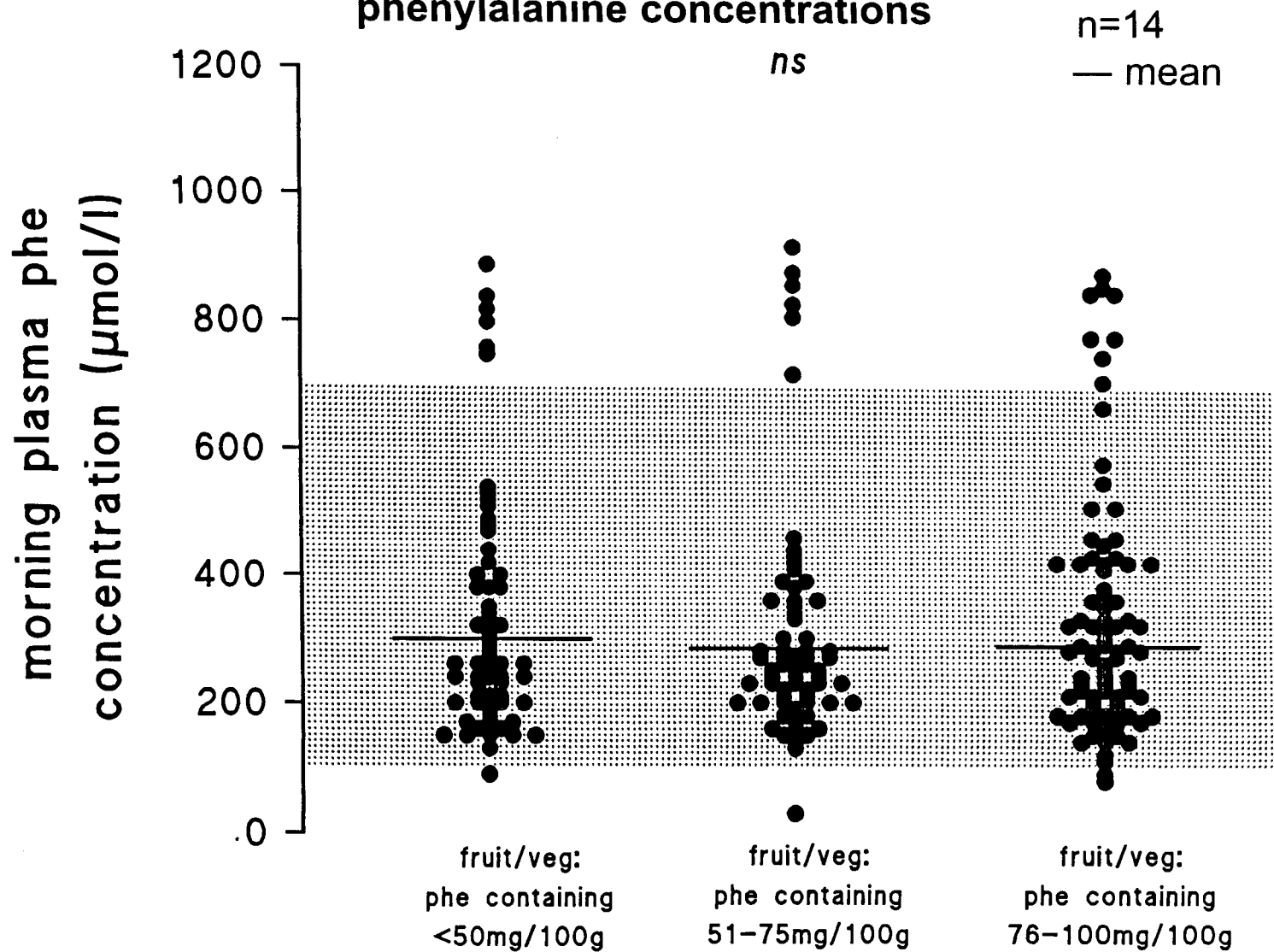
Fig 4.9

Comparison of free use of fruit and vegetables containing phenylalanine <50mg, 51-75mg, and 76-100mg/100g on evening plasma phenylalanine concentrations
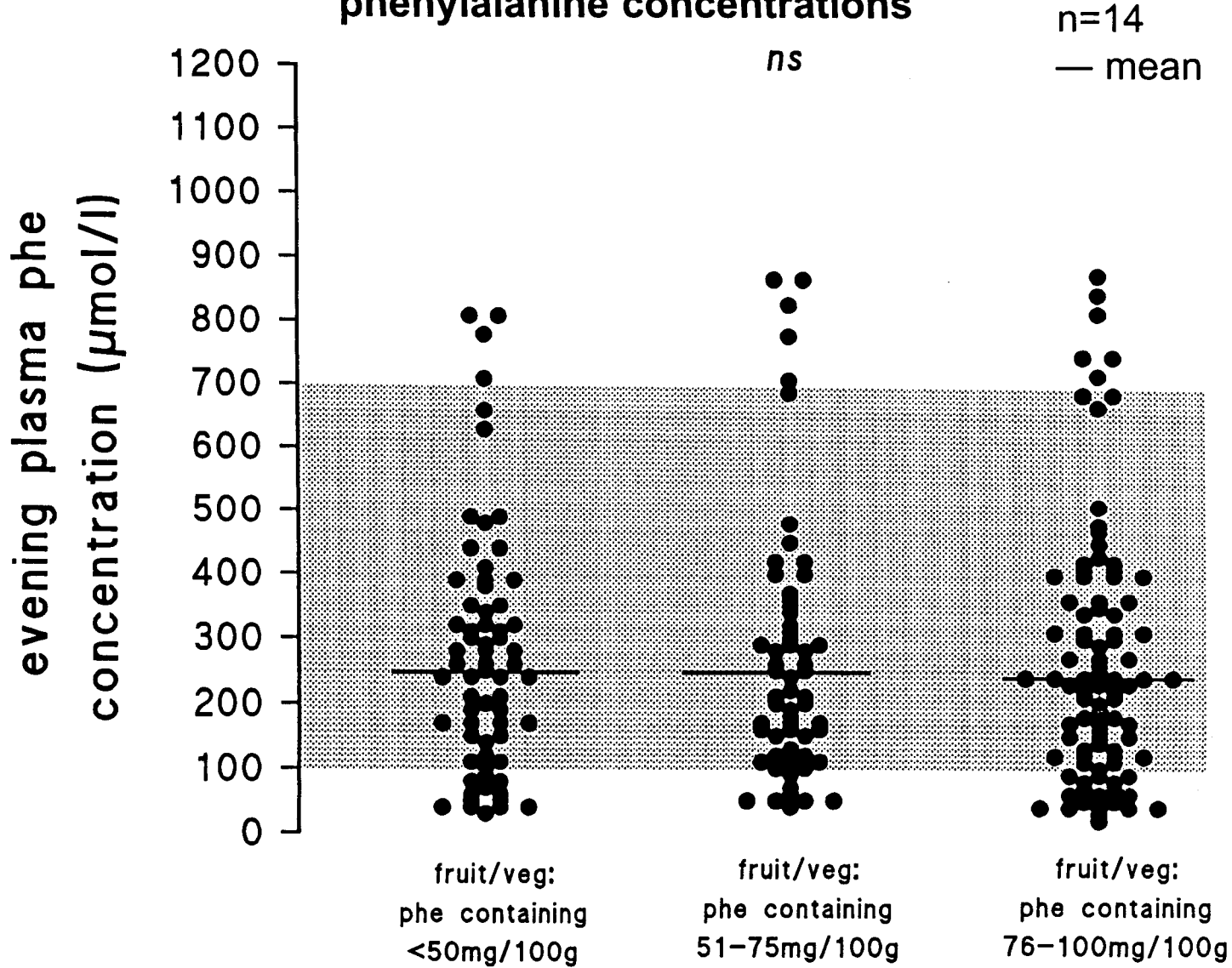
Fig 4.10   **Additional dietary phenylalanine and protein from the free use of vegetables containing phenylalanine 76-100mg/100g**

n=14
— median
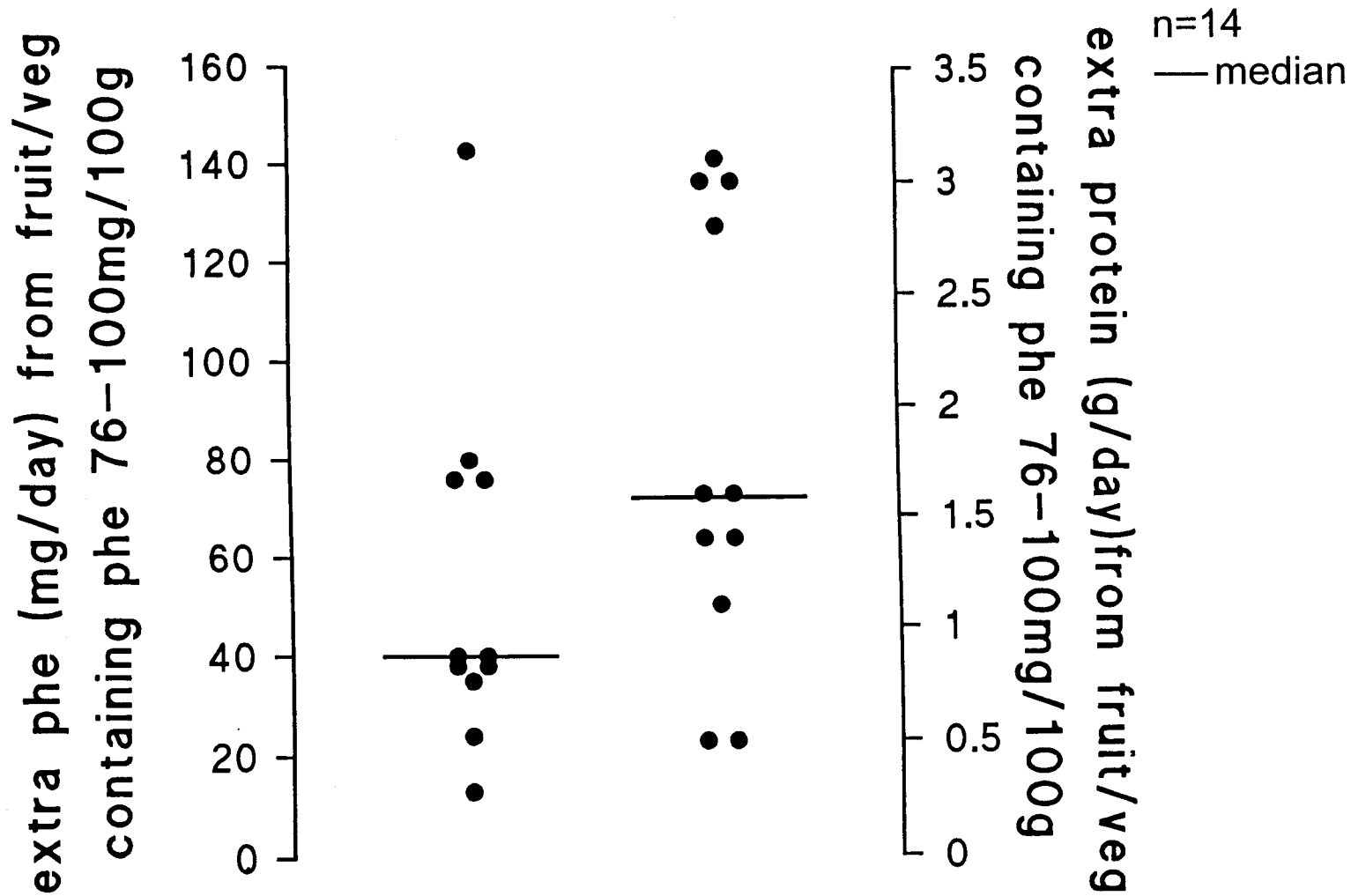
Fig 4.11 Comparison of % excess natural protein from freely allowed foods with fruit and vegetables containing phenylalanine >50mg, 51-75mg and 76-100mg/100g (weeks 1-3 vs. 4-8 ns; weeks 1-3 vs. rest p<0.001; weeks 4-8 vs. rest p<0.05)

n=14

— mean

% extra protein over phe exchanges

fruit/veg:
phe containing
<50mg/100g

week 1-3

fruit/veg:
phe containing
51-75mg/100g

week 4-8

fruit/veg:
phe containing
76-100mg/100g

week 9-15
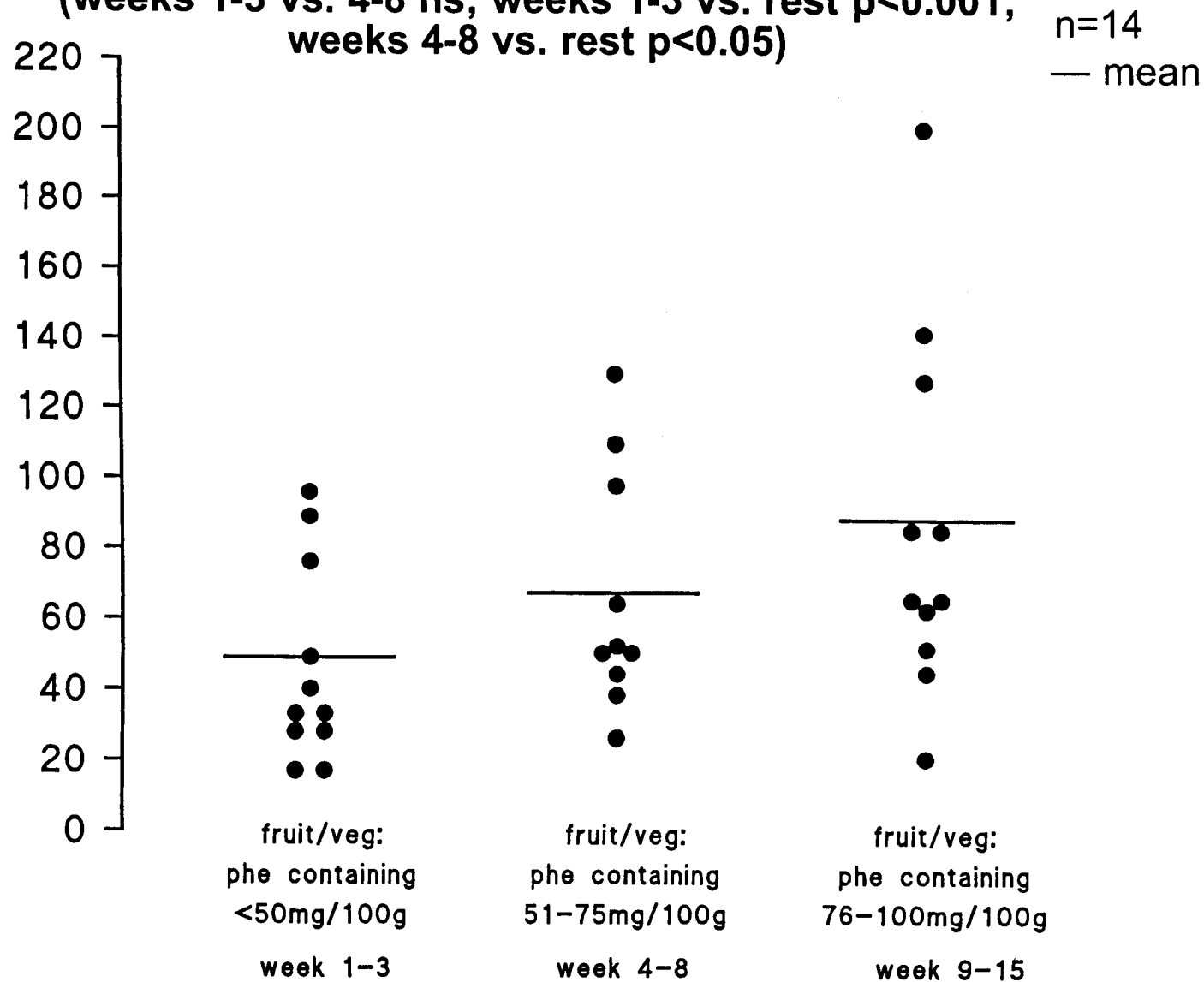
Chapter 5

24 hour plasma phenylalanine variability in PKU

5.1 **Introduction**

There are no data on 24-hour variability of phenylalanine concentrations in children with PKU. Consequently, it is not known what extent a single phenylalanine concentration taken at the same time each day is representative of an entire 24-hour period.

In Chapter 2, large within-patient variations in phenylalanine concentrations were found according to the timing of ingestion and distribution of protein substitute throughout the day. However, that study was limited, in that phenylalanine concentrations were measured at only two set time points within each 24-hour period. The methodology used may therefore have failed to detect considerable and therapeutically important inter- and intra-individual variation in phenylalanine concentrations throughout the remainder of the 24-hour period. The purpose of this study was to investigate in detail the circadian rhythm of plasma phenylalanine in well-controlled children with PKU. The aims were to identify a) the highest and lowest phenylalanine concentrations within a 24-hour period; b) to determine the length of time for which plasma phenylalanine concentrations are outside the recommended MRC Working Group (MRC, 1993b) range; and c) to investigate the extent a single value was representative of an entire 24-hour period. It is possible that extra 24-hour phenylalanine data may alter clinical decisions on dietary management.

5.2 **Subjects and methods**

5.2.1 **Subjects**

Sixteen patients (fifteen Caucasian and one Afro-Caribbean) with classical PKU were recruited. There were three inclusion criteria; 1) maintenance of plasma phenylalanine

concentrations (weekly, fortnightly or monthly according to age group) within the ranges recommended by the MRC Working Group (MRC, 1993b) for 70% of the six months before entering the study; **2)** over 1 year of age; and **3)** parental ability to take skin puncture blood specimens at home by thumb prick. There were 12 girls and 4 boys, with a median age of six years (range 1 to **18** years).

The median number of 50 mg phenylalanine exchanges allocated was six per day (range 3.5 to 18) with an equivalent median natural protein intake of 6g daily. The following amounts of total protein/kg/day from protein substitute and phenylalanine exchanges were allocated:- children 1 year of age: **3.0g/kg;** 2–5 years: 2.5gkg; 6–10 years: 2.0g/kg; over 11 years of age: 1.5g/kg. The brand of protein substitute used was chosen by the patient and was administered either as a paste or drink. Nine patients used XP Maxamaid (Scientific Hospital Supplies), six used Phlexy 10Drink Mix (Scientific Hospital Supplies) and one used Aminogran Food Supplement (UCB Pharma).

The study was approved by the Committee on Medical Ethics of South Birmingham Health Authority. Informed consent was obtained from all parents and from all competent patients.

5.2.2 Methods

Assessment of plasma phenylalanine concentrations

Patients attended the Children's Hospital for 13 hours during one day. Following application of topical anaesthetic cream, indwelling venous cannulae were inserted and kept open throughout the study period by intermittent insertion of heparinised saline. Blood samples for phenylalanine estimation were collected into heparinised tubes at hourly intervals from

09.00h to 21.00h inclusive. The study was continued at home through the remainder of the 24-hour period, with parents taking heel or thumb serial skin puncture blood specimens at midnight, 03.00h and 06.00h. Blood collection technique had been taught and competence assessed by a specialist nurse. The night-time blood specimens were then collected within four hours of study completion and taken to the laboratory the same day. All the blood specimens were centrifuged upon receipt and resulting plasma samples stored at -20°C until analysed. Plasma phenylalanine concentrations were measured by HPLC as previously described.

The child's usual daily activity was maintained as closely as possible during the day of the study. Children under 10 years took part in school type activities in the morning, were entertained by a clown in the afternoon, and watched videos and television in the evening. Older patients watched videos, listened to music and read magazines throughout the day. Children were free of intercurrent infections on the day of study.

Assessment of phenylalanine, protein substitute and energy intake

Customary food intake was maintained as closely as possible on the day of study. Patients ate their usual breakfast at home prior to their first blood test. Packed lunches were provided, and later, an evening meal similar to what they would have at home was eaten. All food and drink intake was documented during the study, including any taken prior to the first blood test of the study. Food to be eaten was weighed using Salter electronic scales (accurate to 5g), about which parents or older subjects had been instructed. Parents recorded only food and drink actually consumed; remaining food was re-weighed and deducted from the total start weight. The weighing and recording of food and drink during the day was

supervised. Timing and quantity of protein substitute consumed was also recorded prospectively.

Nutritional analysis of food intake was calculated using the Microdiet computer programme based on *McCance & Widowson's* *"The Composition of Foods"* (Holland *et al,* 1991), with supplementary analysis data provided by manufacturers and added to the database as described in chapter 2. Energy intake was calculated as a percentage of the EAR (DH, 1991).

5.3 Statistical analyses

Statistical analysis was by Pearson Product Moment Correlation coefficient (r) comparing change in phenylalanine concentrations with timing of protein substitute intake; One-way Analysis of Variance and Tukey-Kramer Multiple Comparisons Test to compare nutrient intake between meal times.

5.4 Results

5.4.1 Plasma phenylalanine concentrations

There was a wide variation in plasma phenylalanine concentration over the time period studied (Fig 5.1, 5.2 and 5.3). The median (range) variations in plasma phenylalanine concentrations (µmol/l) were as follows:

1–5 year olds   150(110-220)   n = 5

6–10 year olds   180(100–280)   **n = 4**

11-18 year olds   170(80-220)   n = 7

There was no correlation between age and change in plasma phenylalanine concentrations. The highest phenylalanine concentration in most children occurred in the morning and *63%* attained their highest level between 06.00h and 09.00h (Fig 5.4). However, in 31% the highest concentration did not occur until after midday and in 19% the peak occurred in late afternoon. In contrast, almost all patients had their lowest phenylalanine concentrations after midday, 63% reaching their lowest point between 18.00 hours and midnight (Fig 5.4).

The first plasma phenylalanine concentration at 09.00h did not reflect the overall pattern of control for the entire 24-hours studied in 44% (n = **7**) of the subjects. In 25% (n = 4) of subjects plasma phenylalanine concentrations were within recommended guidelines at 9.00 h, but mean concentration were below 100 μmol/l for the rest of the 24-hours. In 19% (n = 3) of the subjects, 9.00 h plasma phenylalanine concentrations were higher than recommended guidelines but mean concentrations were within or almost within recommended guidelines for the overall time period. The 24-hour mean phenylalanine concentration (292 μmol/l; SD 226) was significantly less than the mean first blood phenylalanine concentration taken at 9.00 h (338 μmol/l; SD 195) (p <0.001). Only in two subjects were the 9.00h plasma phenylalanine concentrations lower than the 24-hour mean. Individual early morning and mean phenylalanine concentrations are outlined in Table 5.1 and Fig 5.5.

Phenylalanine concentrations of less than 100μmol/l occurred in 46% of children aged 10 years or below (n = 5/11). The length of time the phenylalanine concentration remained below 100μmol/l varied from **50–77%** of the 24-hour period studied. Three children had concentrations of less than 30 μmol/l for 2, 6 and 7 hours, but in each of these phenylalanine

Table 5.1

First early morning plasma phenylalanine concentration compared with mean 24-hour plasma phenylalanine concentrations

Subject no.	9.00 a.m. phenylalanine concentration (μmol/l)	Mean daily phenylalanine concentration (μmol/l)
1	100	49
2	250	180
3	260	234
4	130	54
5	300	265
6	140	97
7	300	282
8	170	85
9	620	459
10	150	115
11	330	323
12	420	373
13	730	677
14	290	246
15	710	626
16	840	741

concentrations were over 100μmol/l at 9.00 a.m. in the morning. In these children, the median phenylalanine for the day was 65 μmol/l. Only one child of 10 years or under had phenylalanine concentrations above the MRC range guidelines (MRC, 1993b) (for 27% of the time period studied), but 3 of 5 subjects over 11 years had phenylalanine concentrations above the MRC guidelines for 24% of the time period studied. Daily changes in plasma phenylalanine concentrations are given in Table 5.2.

5.4.2 Dietary intake

Neither the energy or total protein intake correlated with the change between highest and lowest plasma phenylalanine concentrations in 24 hours (Fig 5.6 and 5.7). The median (range) energy intake was 117% (60–208%) of the EAR (DH, 1991). More energy was consumed at lunch and evening than breakfast (breakfast vs lunch p <0.01 ; breakfast vs. evening **p** <0.01) (Fig 5.8). Equally more carbohydrate was consumed in the evening than breakfast (p <0.05) (Fig 5.9), and more fat was consumed at lunch and evening than breakfast (**p** <0.05) (Fig 5.10).

The median (range) total protein intake was 230% (151–321%) of the RNI (DH 1991). The subjects consumed a median (range) excess of 36% (-15–183%) natural protein from free foods in addition to their phenylalanine exchanges. There was no correlation with excess protein intake and change between highest and lowest blood phenylalanine concentrations (Fig 5.11). The majority of the natural protein was eaten in the evening with just over a quarter being eaten at breakfast and lunch (breakfast vs. evening p<0.01 ; lunch vs. evening p <0.05) (Fig 5.12). Thirty one percent (n = 5/16) of subjects ate more than 50% of their natural protein intake in the evening. However, this had no significant effect on blood

Table 5.2

Age, phenylalanine exchanges, daily changes in plasma phenylalanine concentrations.

Age	No. of phenylalanine exchanges	Daily change in plasma phenylalanine concentrations (μmol/l)
1	5	147
1	5	180
3	12	110
3	5	220
4	6	150
5	5	160
5	5	100
6	6	196
6	3.5	280
8	5	140
8	6	170
12	11	220
12	6.5	150
14	11	80
16	14	130
18	18	180

phenylalanine concentrations within 1 to 2 hours of mealtime consumption. The 24-hour

blood phenylalanine profile of each of these **5** patients is given in Fig 5.13.

The timing of protein substitute intake was the only factor that affected change in plasma

phenylalanine concentrations. The greater the quantity of protein substitute consumed by the

16.00h specimen, the larger the decrease in daytime phenylalanine concentration

$(r = -0.5711)$ $(p < 0.05)$ (Fig 5.14). The less protein substitute consumed after 16.00h, the

larger was the increase in plasma phenylalanine concentration between 16.00h to 06.00h the

following morning $(r = 0.6516)$ $(p = 0.0062)$ (Fig 5.15). Sixty three percent (n = 10) of the

subjects took the protein substitute in 3 doses, split between morning, lunch/afternoon and

evening; 25% (n = 4) took the protein substitute in 2 doses; and 12% (n = 2) took the protein

substitute in 4 doses throughout the waking hours. Slightly more protein substitute was

taken at breakfast than at lunch and evening meal, but the difference was not significant (Fig

5.16).

5.5 Summary

In conclusion, there is wide variability in phenylalanine concentrations throughout the day in

children with PKU. A single early morning blood phenylalanine concentration does not

predict quality of phenylalanine control in PKU and in 44% of subjects it gave misleading

information about overall 24-hour phenylalanine control. In this study the median difference

between highest and lowest concentrations observed was 155 µmol/l, with peak

concentrations usually occurring before midday, and lowest in the afternoon or evening.

Although only 16 patients were studied in a single 24-hour period they represented a wide

age range and all had classical PKU. The main dietary factor that appeared to be responsible for the variable phenylalanine concentrations was the uneven distribution of protein substitute during daytime hours. Adjusting the timing of protein substitute intake may lead to more stable 24-hour plasma phenylalanine profiles in PKU and ensure early morning plasma phenylalanine concentrations give a more accurate picture of overall phenylalanine control.

Fig 5.1

24h plasma phenylalanine profile of
1-5 year children with PKU

plasma phe concentration (μmol/l)

time (h)

09.00

06.00

129
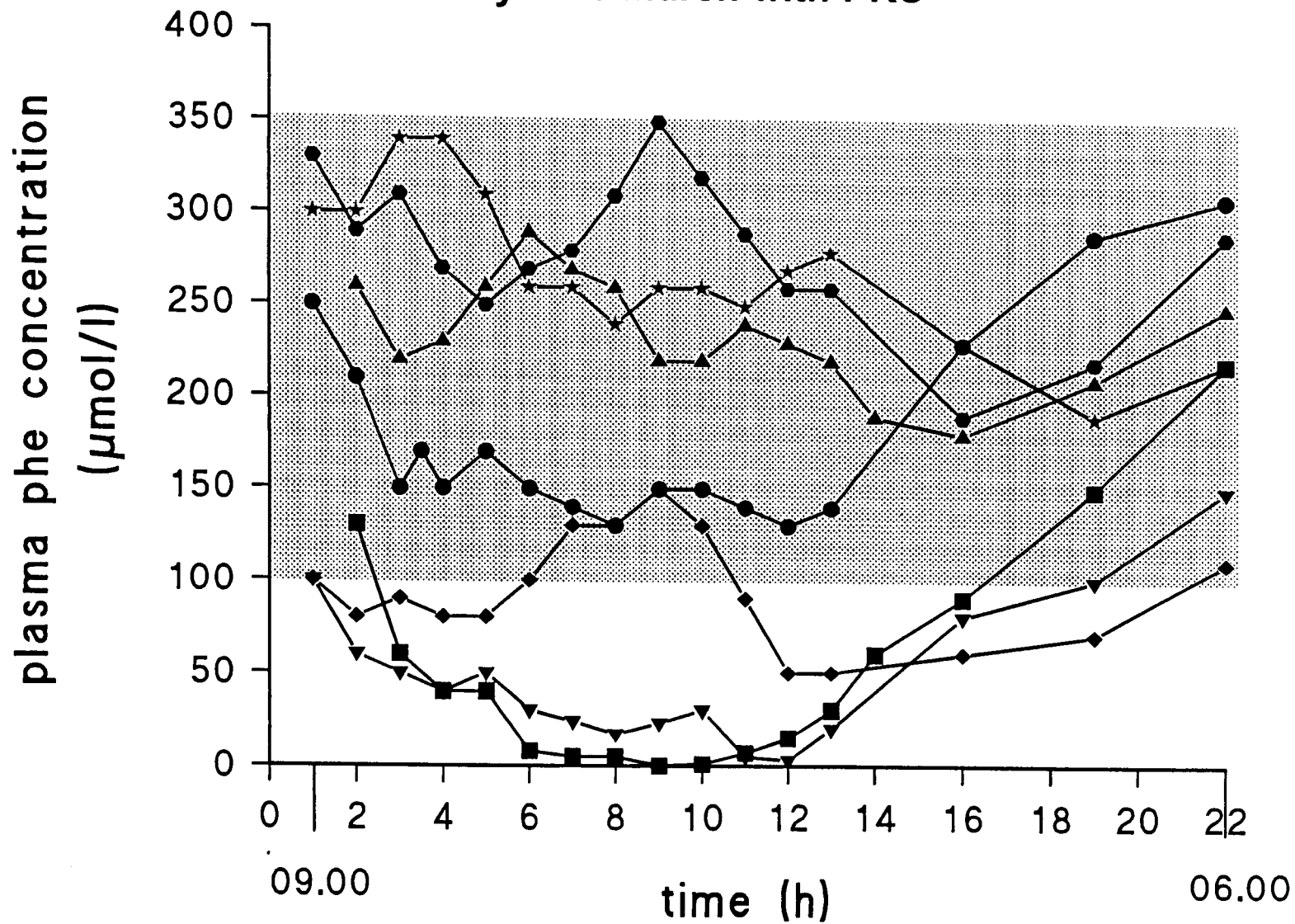
Fig 5.2

24h plasma phenylalanine profile of 6-9 year children with PKU

plasma phe concentration (µmol/l)

700
600
500
400
300
200
100
0

0 2 4 6 8 10 12 14 16 18 20 22

09.00

time (h)

06.00
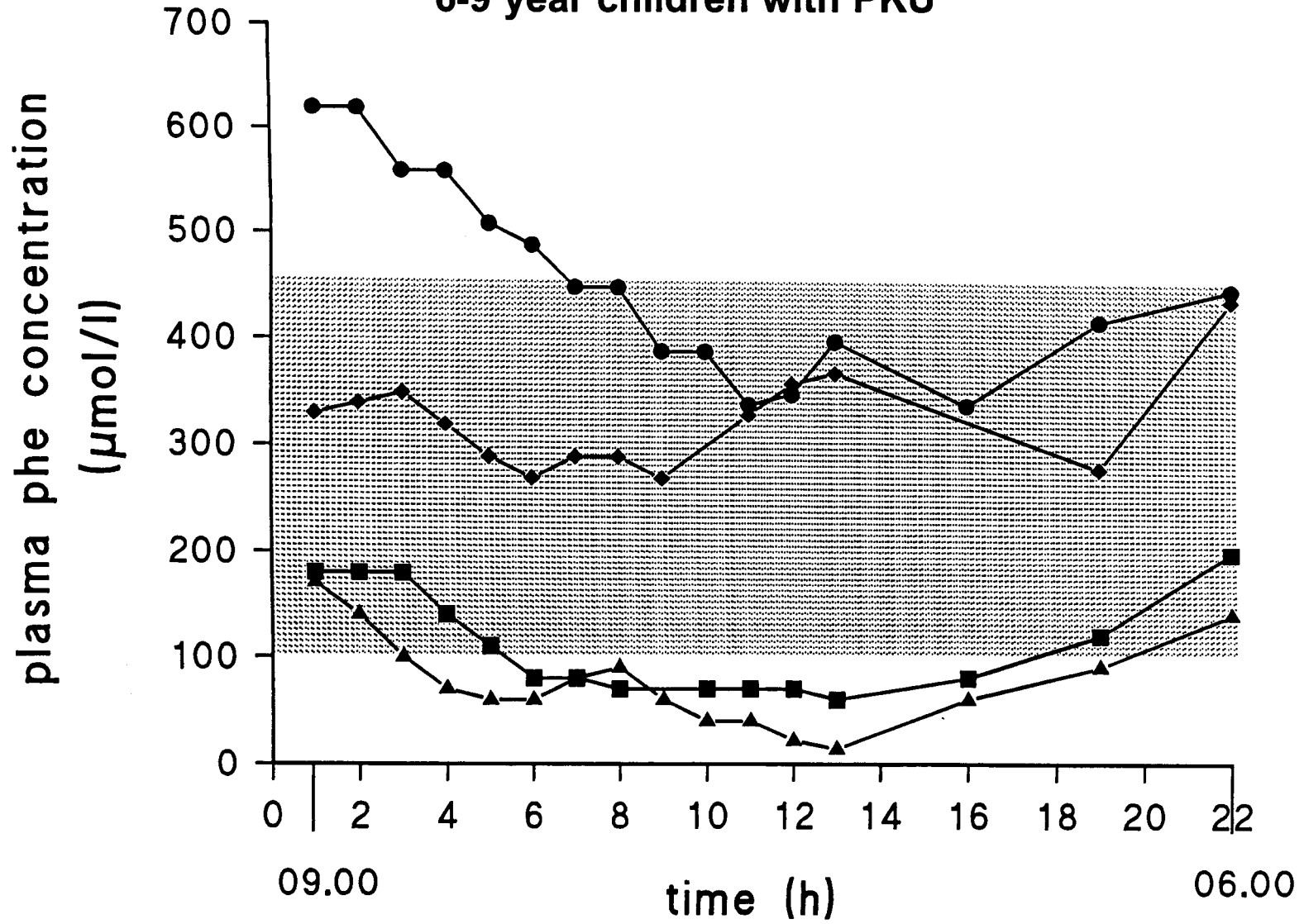
Fig 5.3

**24h plasma phenylalanine profile of
>11 year children with PKU**

plasma phe concentration (μmol/l)

time (h)

09.00   06.00
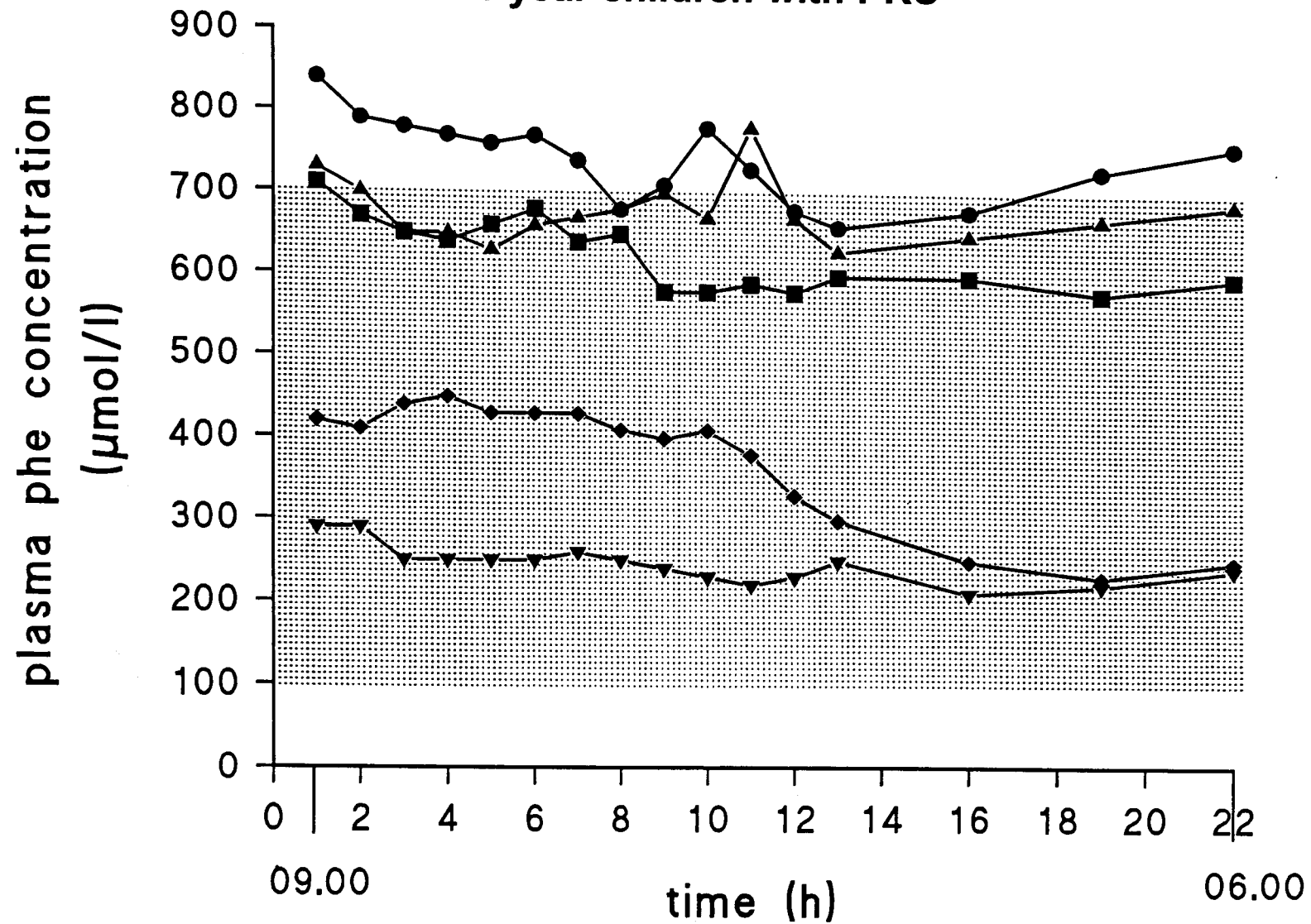
Fig 5.4

Highest and lowest phenylalanine concentrations over 24h
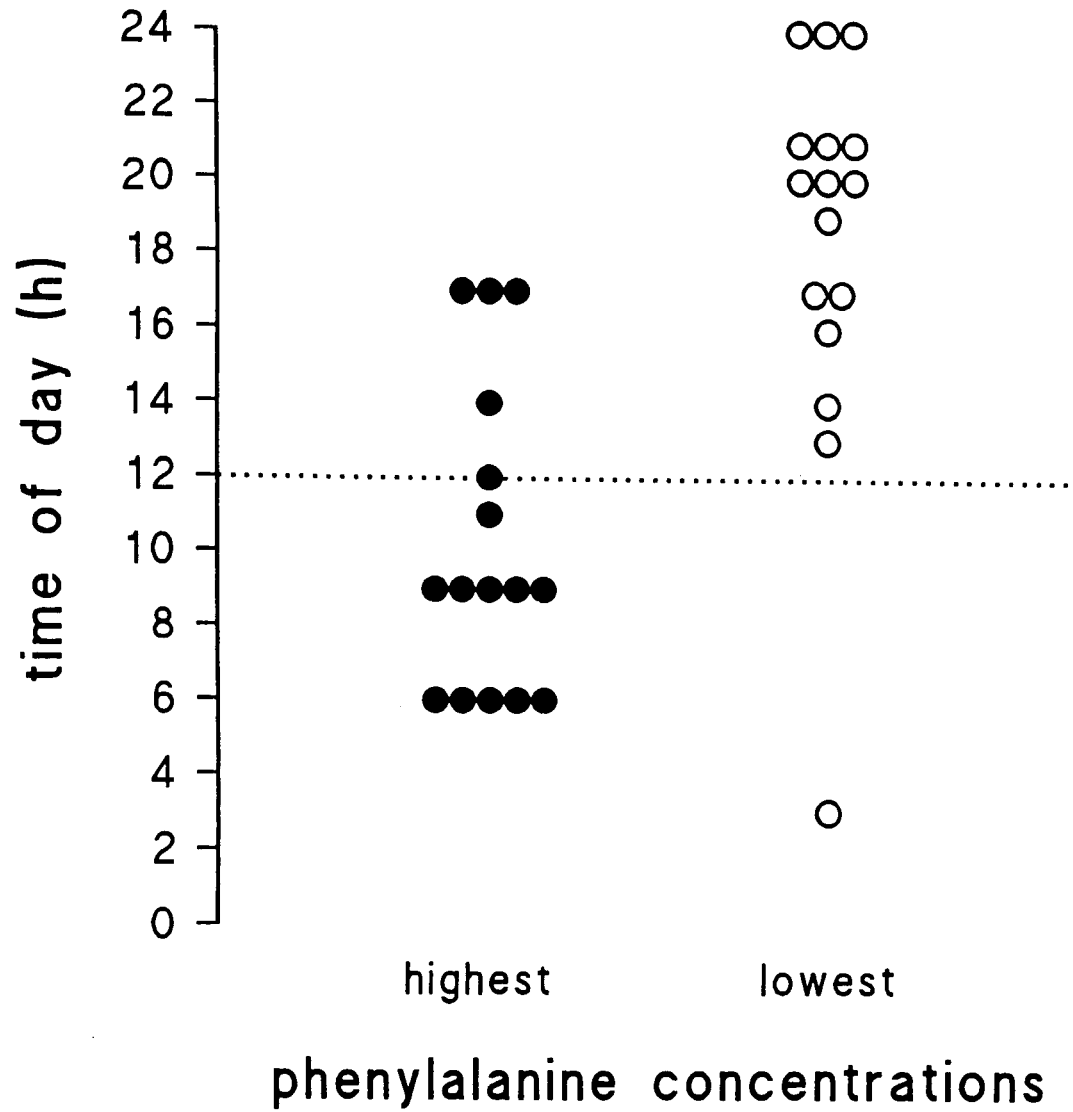
Fig 5.5   **Comparison of 9.00am compared with mean of 24 hour plasma phenylalanine concentrations**
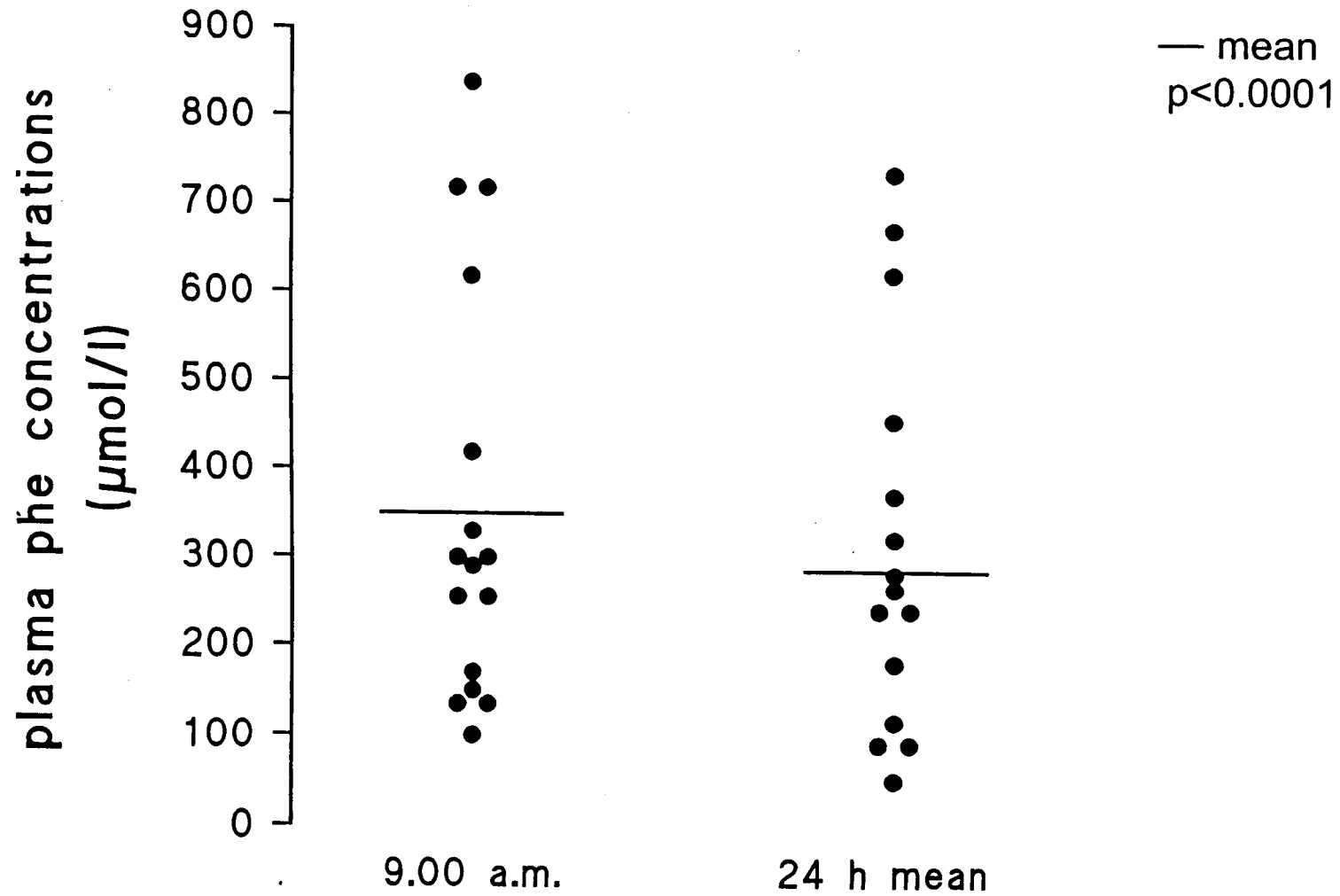
Fig 5.6   Correlation of the change between highest and lowest plasma phenylalanine concentrations and % energy intake of EAR

r= -0.08
p= 0.7649

change between highest and lowest plasma phe (μmol/l)

energy intake (% of EAR)
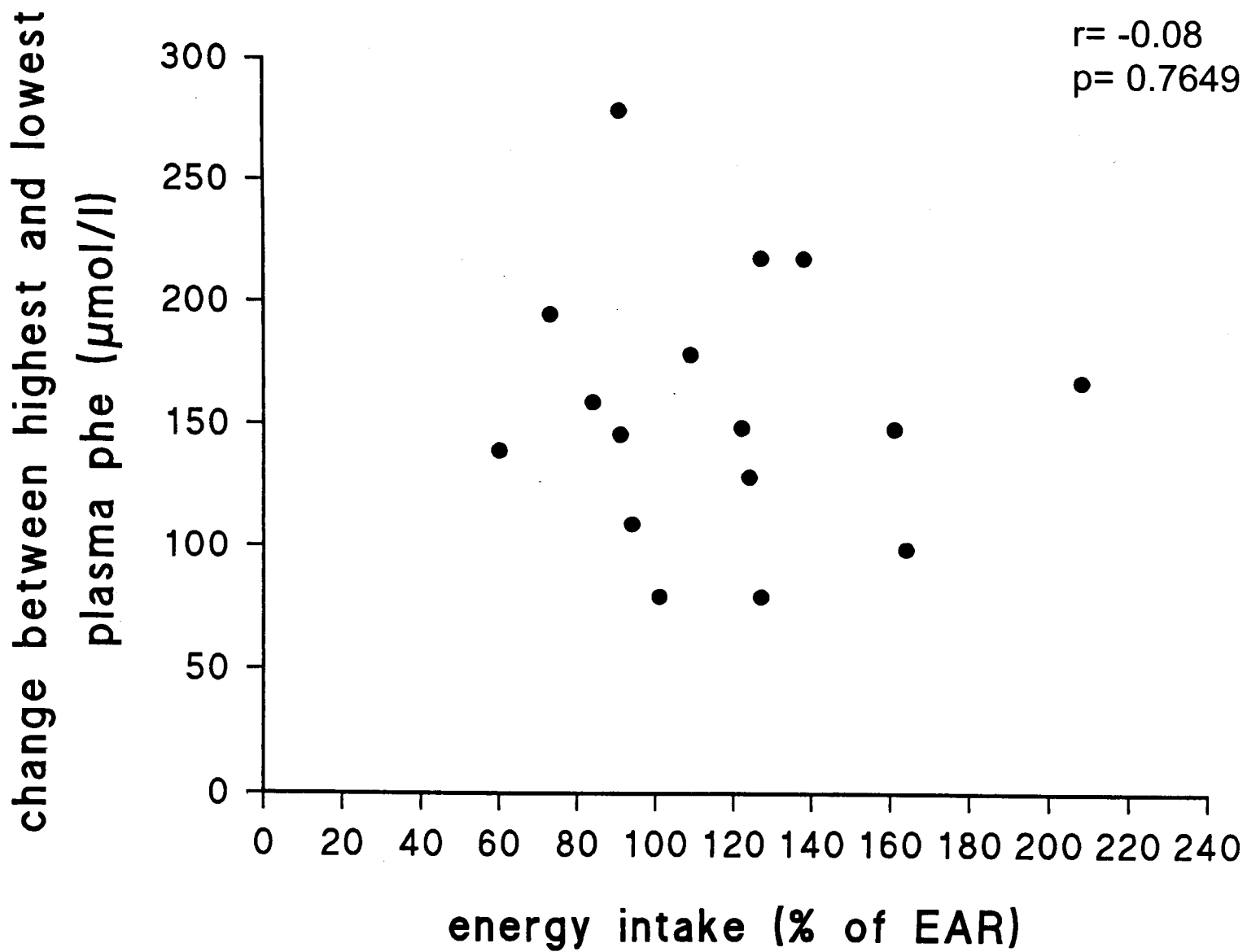
Fig 5.7   **Correlation of the change between highest and lowest plasma phenylalanine concentrations and protein intake expressed as % of RNI**

r= 0.373
p= 0.1547
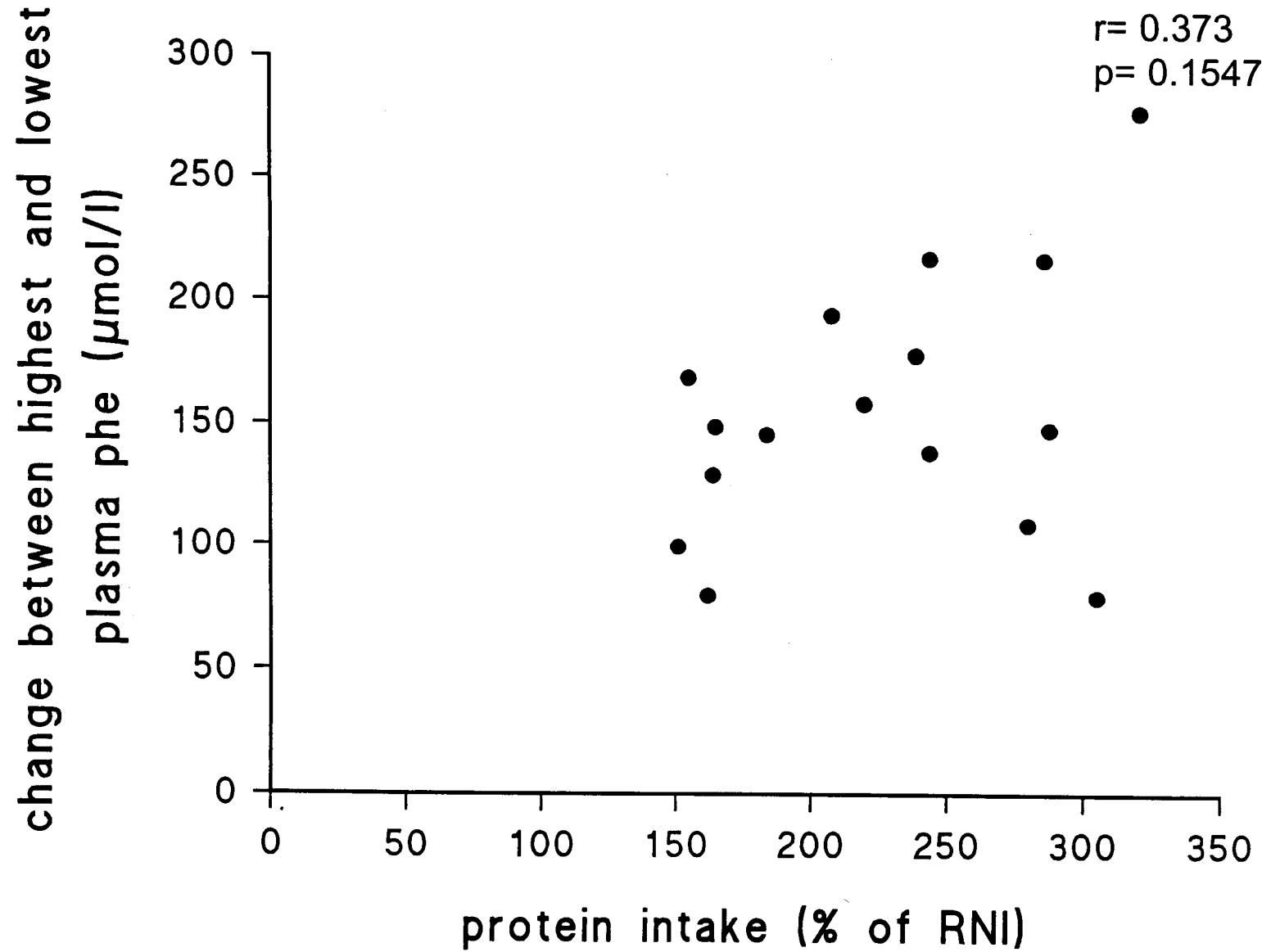
Fig 5.8

% distribution of energy intake throughout the day

— mean
a vs b p<0.001
a vs c p<0.01
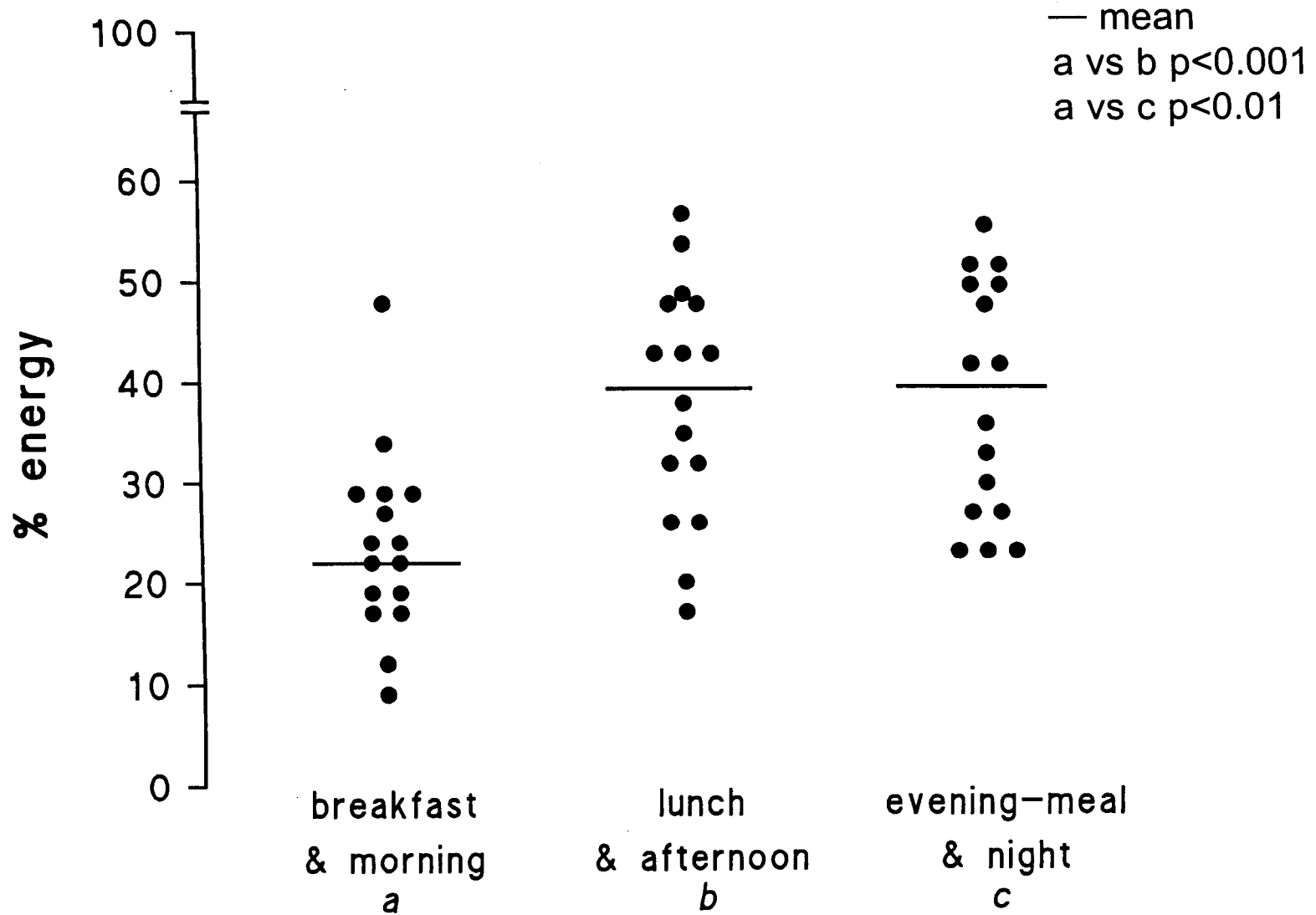
Fig 5.9

% distribution of carbohydrate intake throughout the day

— mean

a vs c p<0.05

137
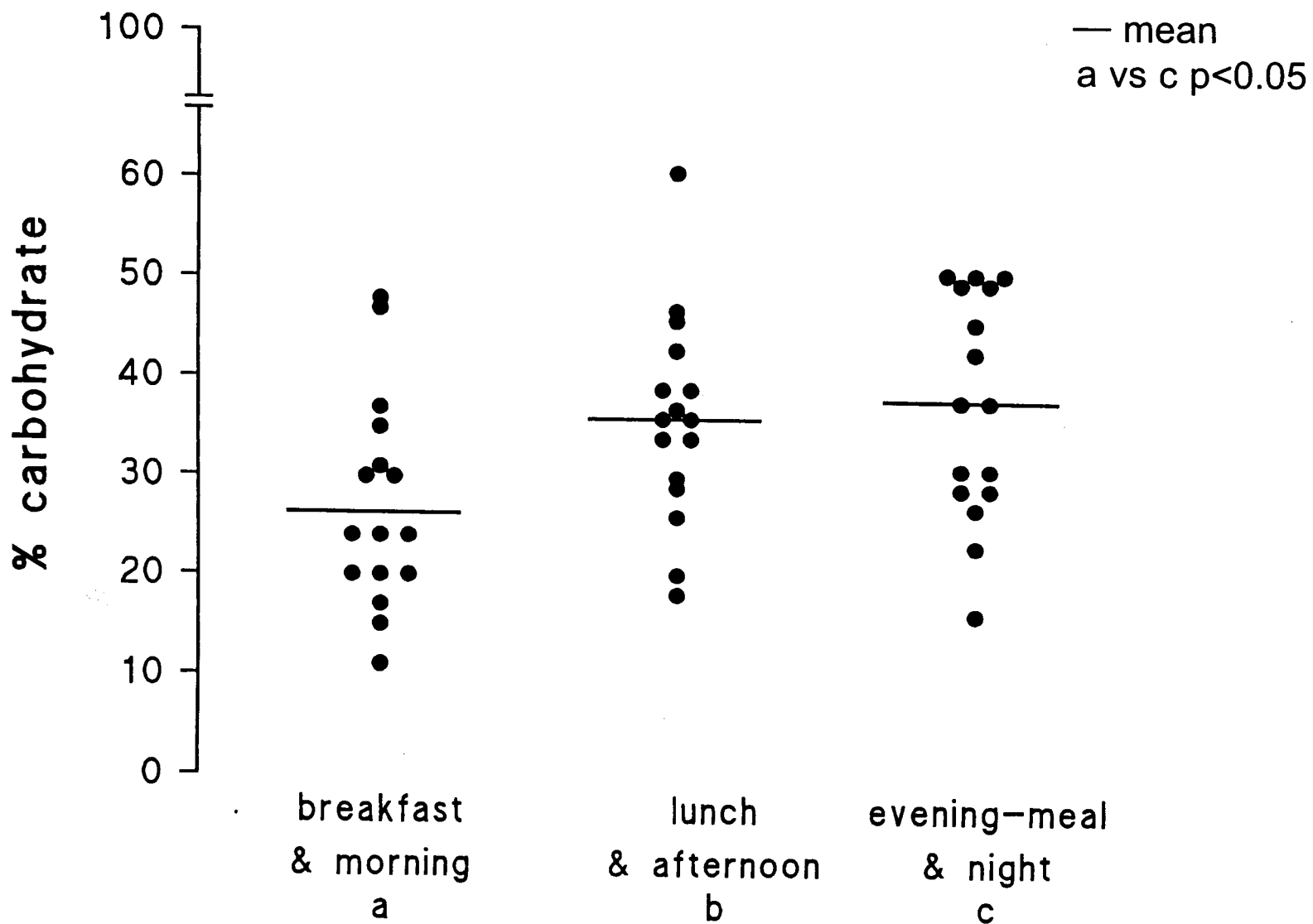
Fig 5.10

% distribution of fat intake throughout the day

— mean
a vs b p<0.05
a vs c p<0.05
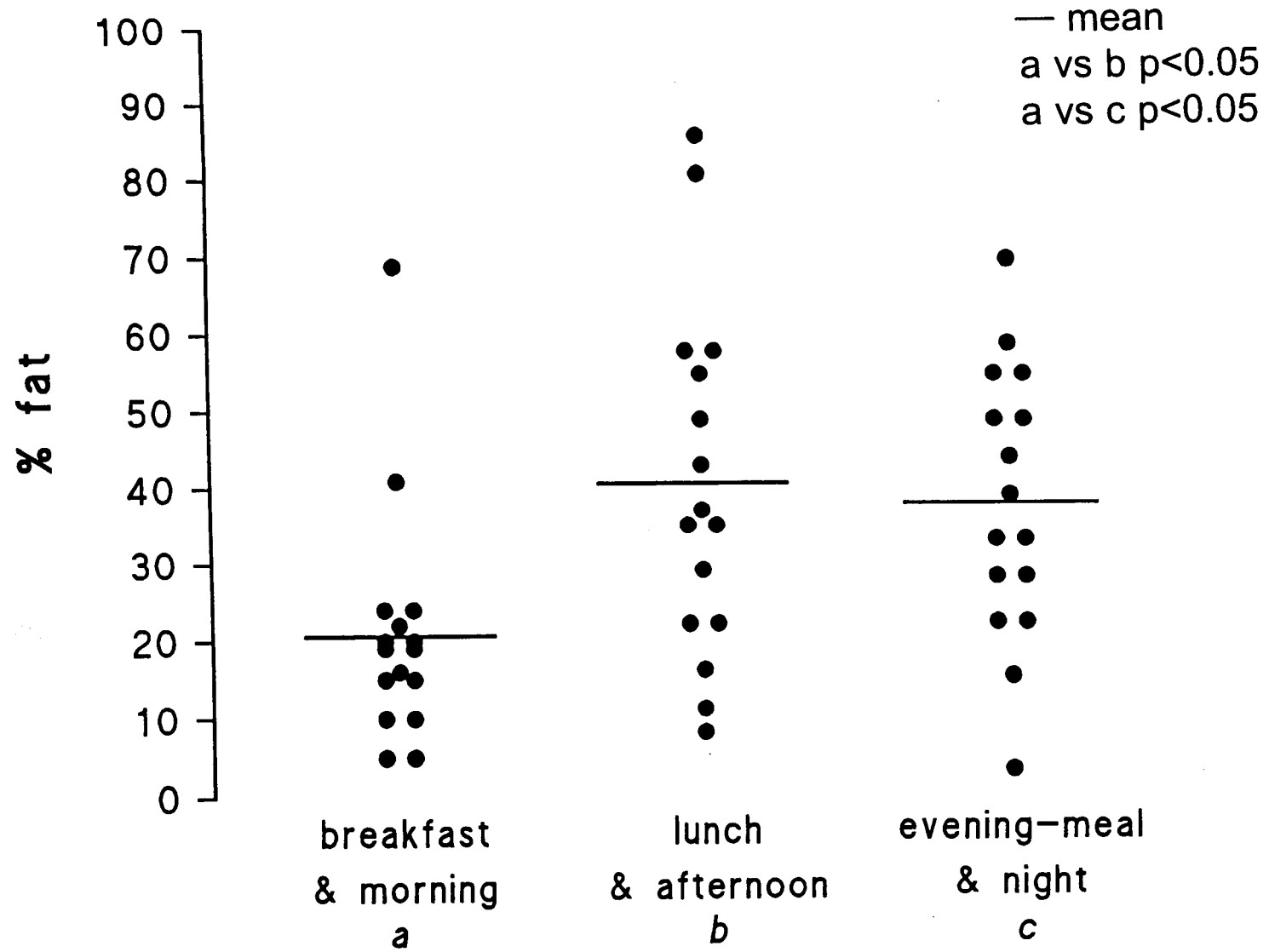
Fig 5.11 Correlation of the change between highest and lowest plasma phenylalanine concentrations and excess protein intake over allocated exchanges
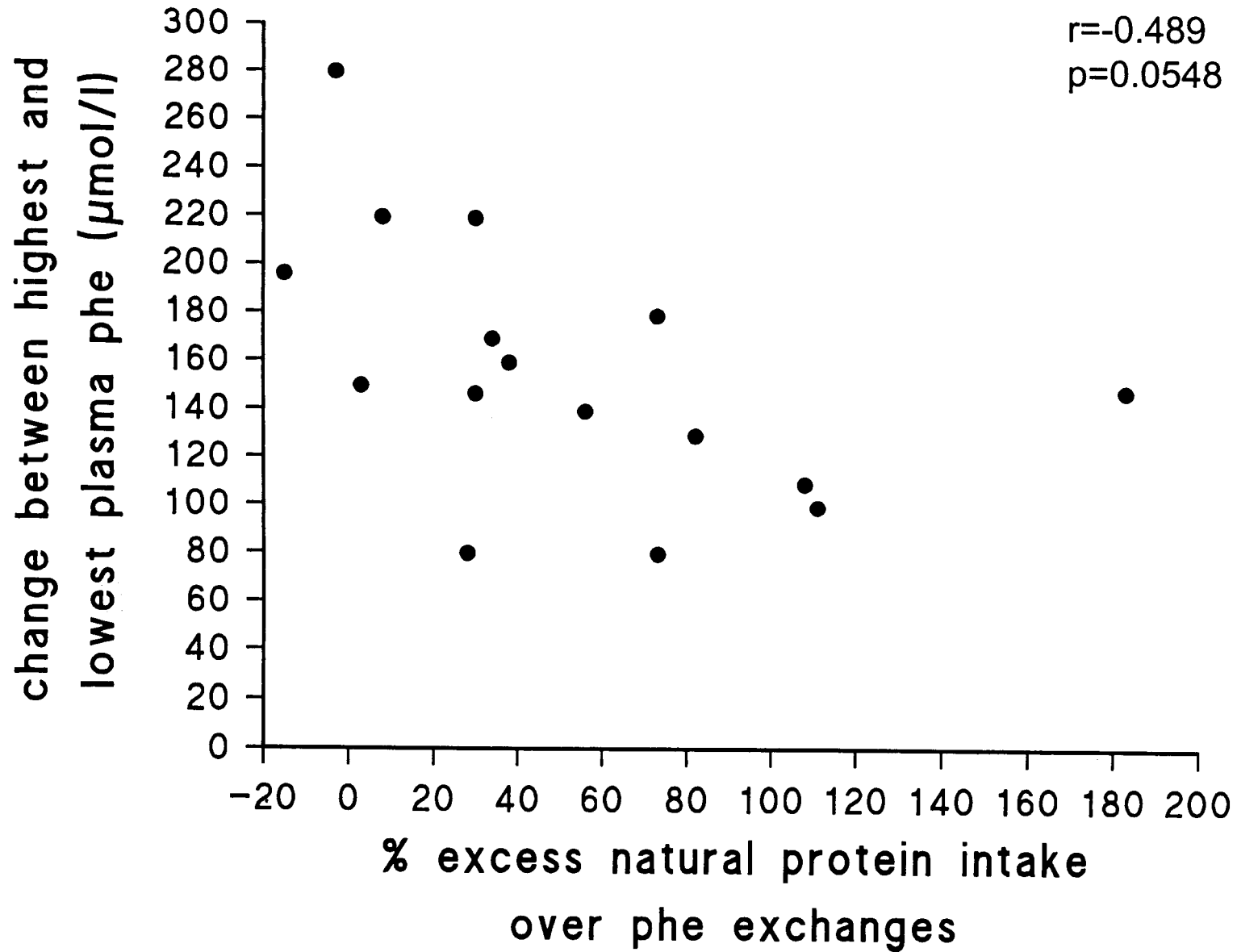
Fig 5.12

% distribution of protein intake throughout the day

— mean
a vs c p<0.01
b vs c p<0.05
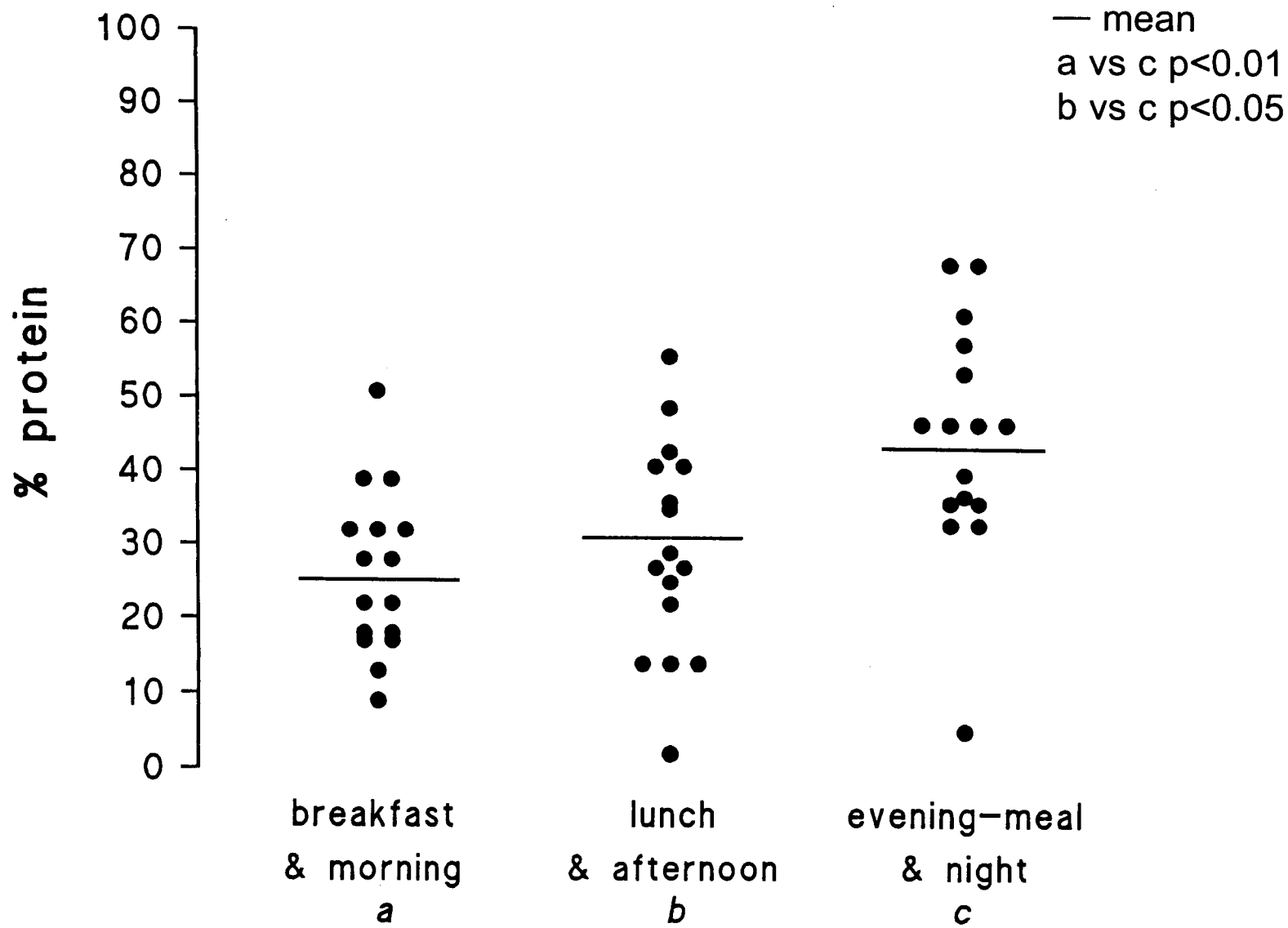
Fig 5.13 **Plasma phenylalanine profiles of five children who consumed over 50% of their natural protein intake at the evening meal**

←=timing of evening phenylalanine intake

Plasma phe concentration (μmol/l)

800
700
600
500
400
300
200
100
0

0 2 4 6 8 10 12 14 16 18 20 22

09.00   06.00

time (h)
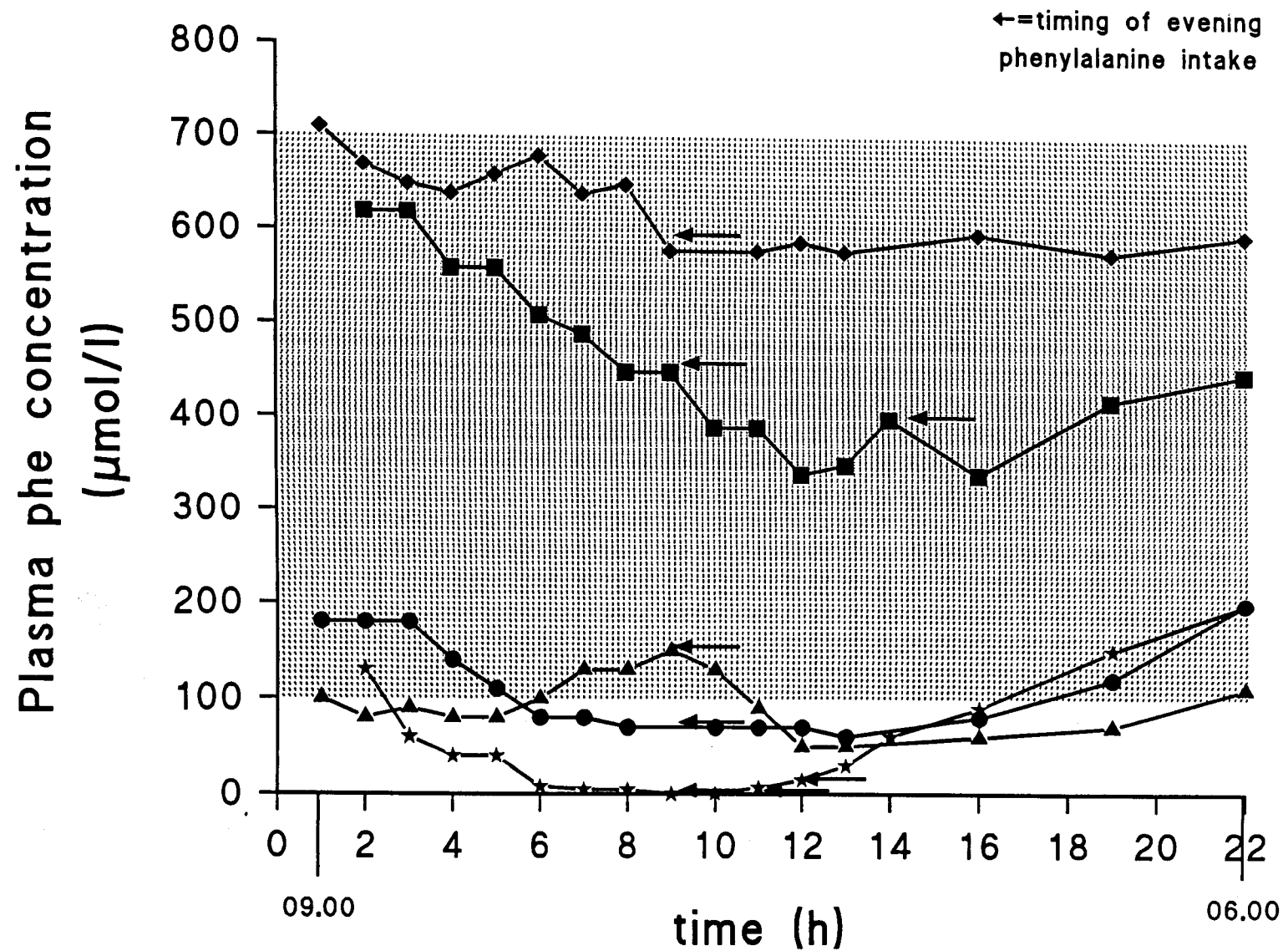
Fig 5.14 **Correlation of the % protein substitute taken by 16.00h and daytime change in plasma phenylalanine concentration**

r=-0.5711
p<0.05

daytime change in plasma phe concentration (μmol/l)

% protein substitute taken by 16.00 h
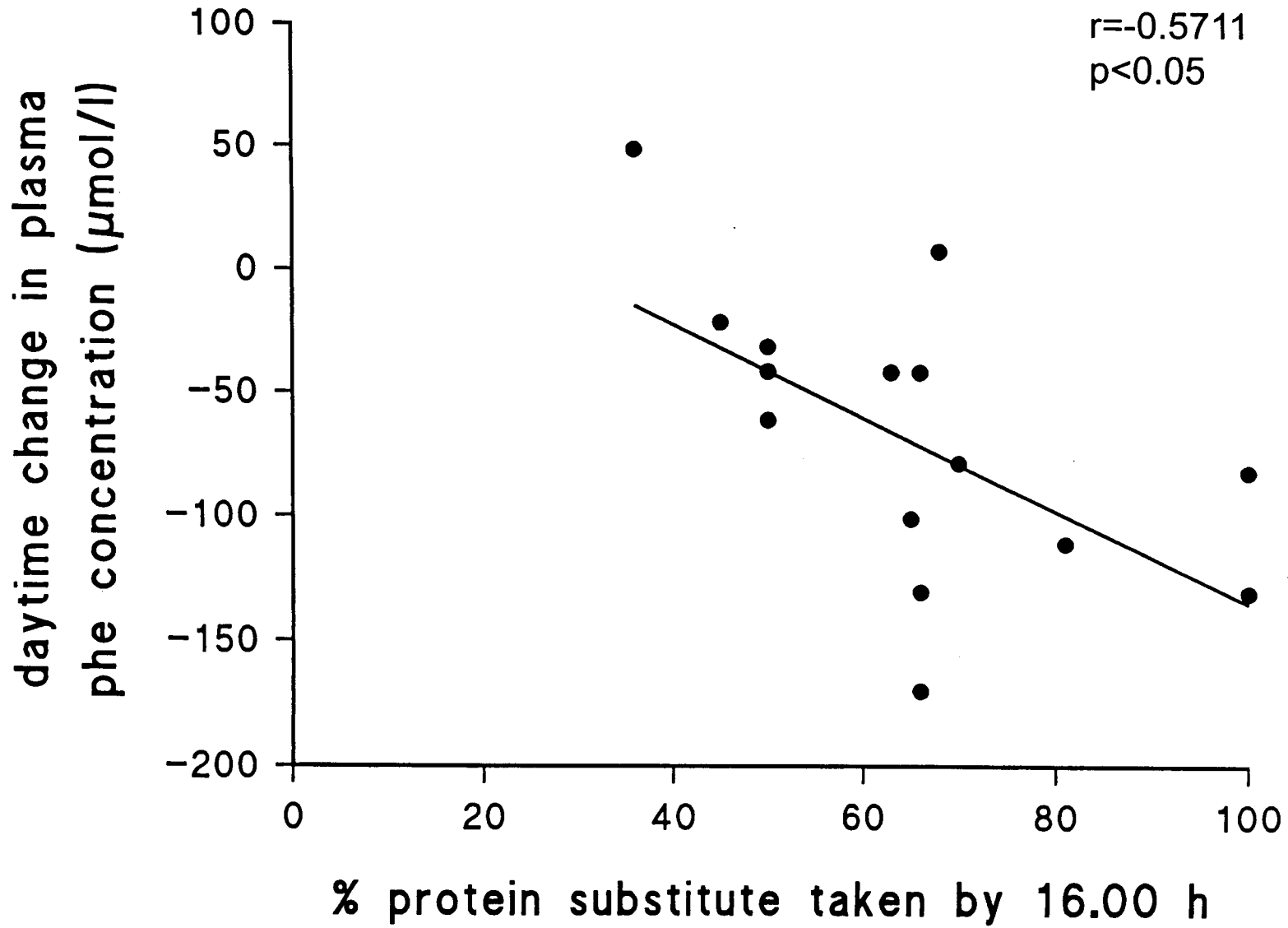
Fig 5.15   **Correlation of the % protein substitute intake taken by 16.00h and the night-time change in plasma phenylalanine concentration**

r=0.6516
p=0.0062
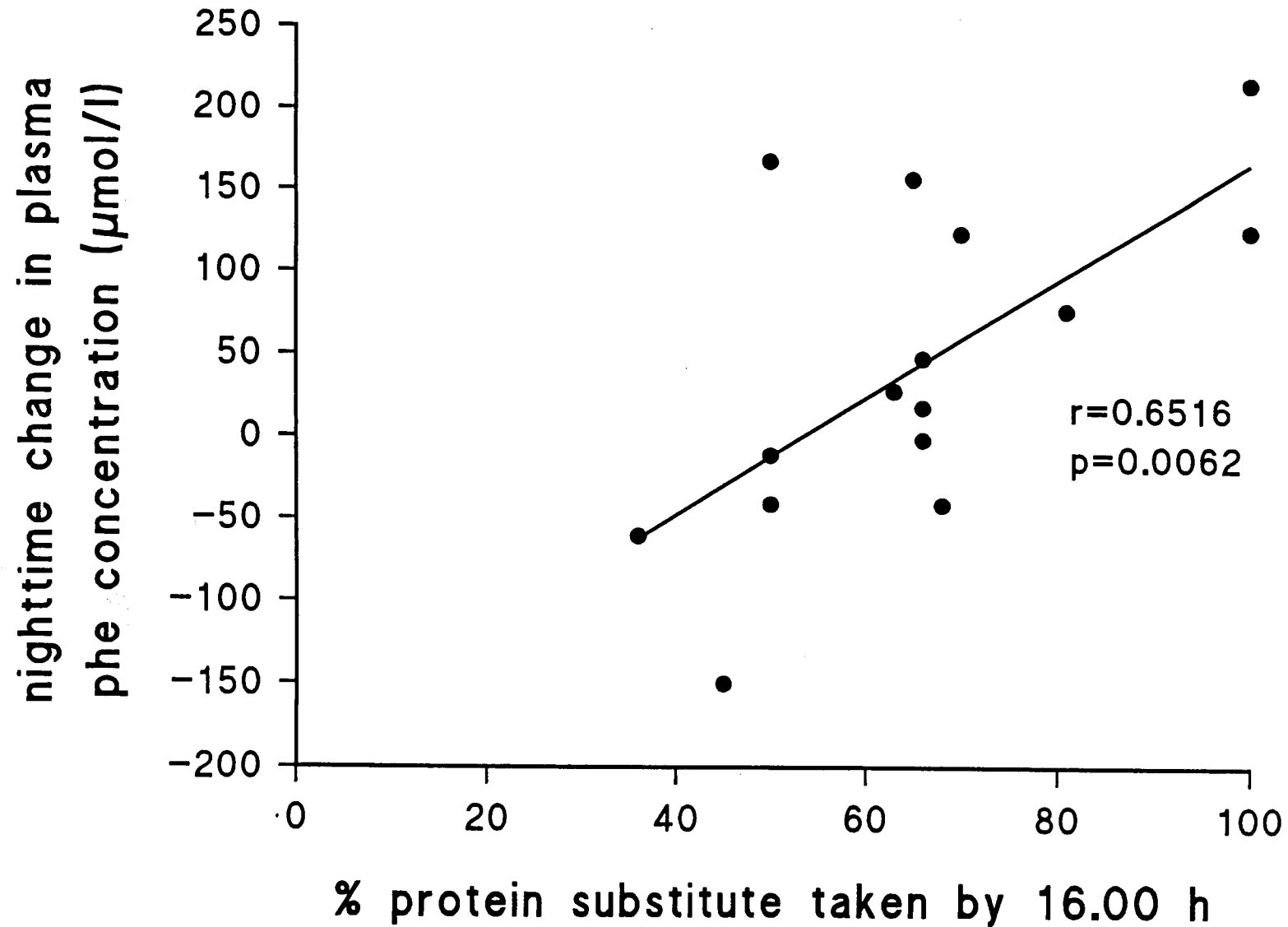
Fig 5.16

% distribution of protein substitute intake throughout the day
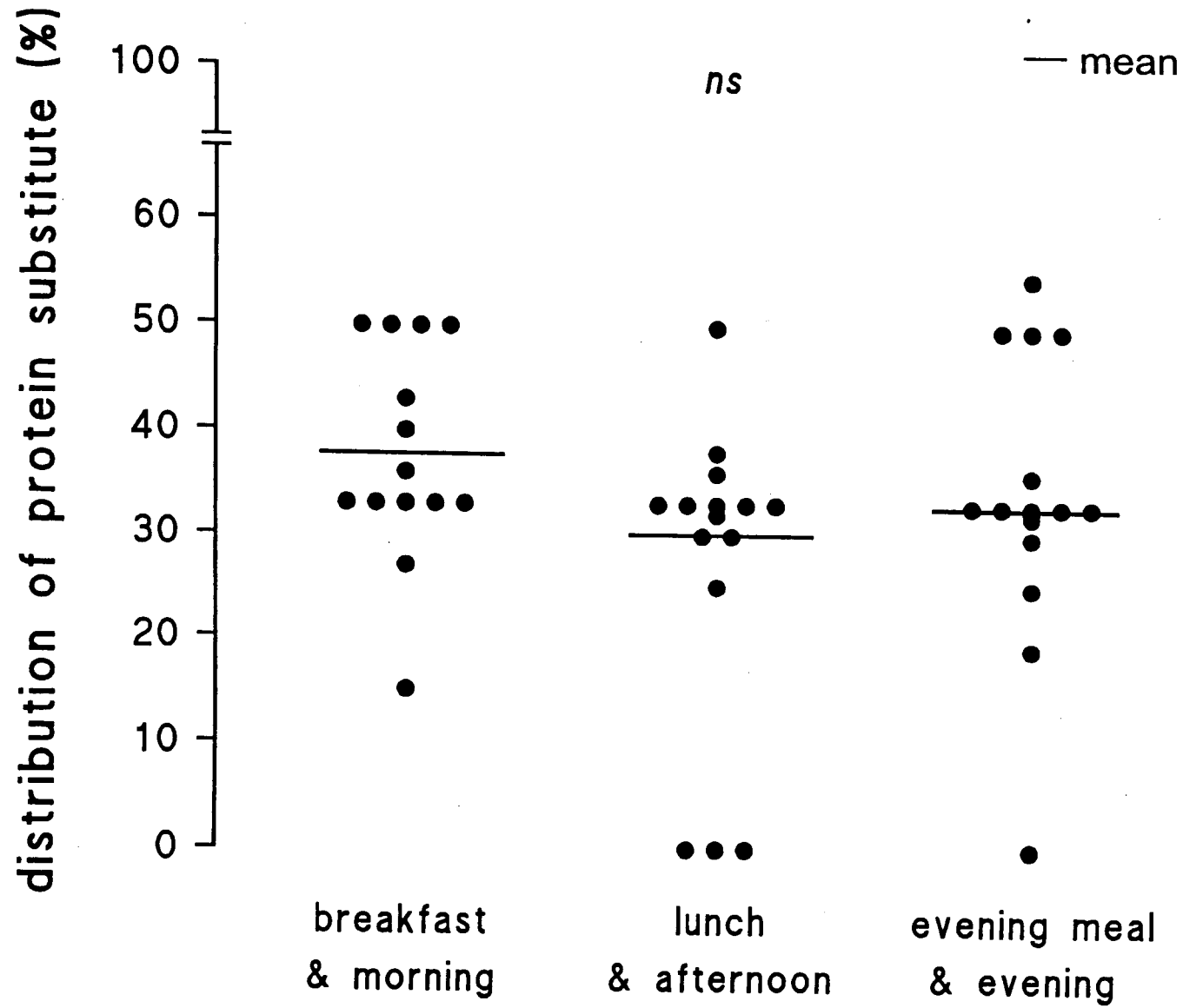
Chapter 6

Part A

The effects of altered timing of administration of protein substitute on variability in plasma phenylalanine concentrations

6.1 Introduction

Plasma phenylalanine concentrations vary widely in children and adolescents with PKU. In Chapter *5,* in the study on 24-hour plasma phenylalanine variability in PKU, the median variation within 24 hours was 155 µmol/l; with peak concentrations usually occurring before midday; lowest concentrations tending to occur in the afternoon or evening. The reasons for this were unclear, but there was some evidence to suggest it was probably due to the uneven distribution of the L-amino acid phenylalanine-free protein substitute.

In this study, the hypothesis that administration of protein substitute over longer daytime hours in more frequent and smaller doses leads to improved control compared with the conventional practice of three doses taken with main meals, was examined. Children with well-controlled PKU (according to current MRC 1993b criteria*) were entered into a randomised, crossover study in which three protocols of protein substitute administration were compared.

6.2 Subjects and methods

6.2.1 Subjects

Fifteen patients (14 Caucasian and 1 Afio-Caribbean) with classical PKU were recruited. There were three inclusion criteria: 1) maintenance of at least 70% plasma phenylalanine concentrations (weekly, fortnightly or monthly according to age group) within ranges

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*   Children 0–5 years maintained blood phenylalanine concentrations between 120–360 µmol/l; school age children 120-480 µmol/l; children >10years 120–700 µmol/l.

recommended by the MRC Working Group (MRC, 1993) in the six months before entering the study; 2) over 1 year of age; and 3) demonstrable parental ability to take heel and thumb prick blood specimens at home. Twelve girls and three boys, with a median age of 4 years (range 1 to 20 years) fulfilled these criteria and agreed to enter the study.

The median number of 50 mg phenylalanine exchanges allocated was six per day (range 3.5–16) with an equivalent median natural protein intake of 6g daily. The following amounts of total protein from protein substitute and phenylalanine exchanges were allocated:- age <2 years: 3.0g/kg/day; 2-5 years: 2.5g/kg/day; 6-10 years: 2.0g/kg/day; over 11 years of age: 1.5g/kg/day.

Patients continued to take their usual brand of protein substitute throughout the study. This was taken either as a paste or a drink. Ten patients used XP Maxamaid (Scientific Hospital Supplies); four used Phlexy 10 Drink Mix (Scientific Hospital Supplies); and one used PK Aid 4 (Scientific Hospital Supplies). XP Maxamaid and Phlexy 10 Drink Mix are based on L-amino acids and supplemented with carbohydrate; PK Aid 4 is based on L-amino acids only.

The study was approved by the Committee on Medical Ethics of South Birmingham Health Authority. Informed consent was obtained from all parents and all competent patients.

6.2.2 Study design (Fig 6.1)

This was a multiple, randomised, crossover study that was conducted at home. Each patient took the protein substitute for a period of 7 days as follows (Figure 6.1):

Fig 6.1   Study design: Percentage dosage and time of administering protein substitute (Protocol A, Protocol B, Protocol C)
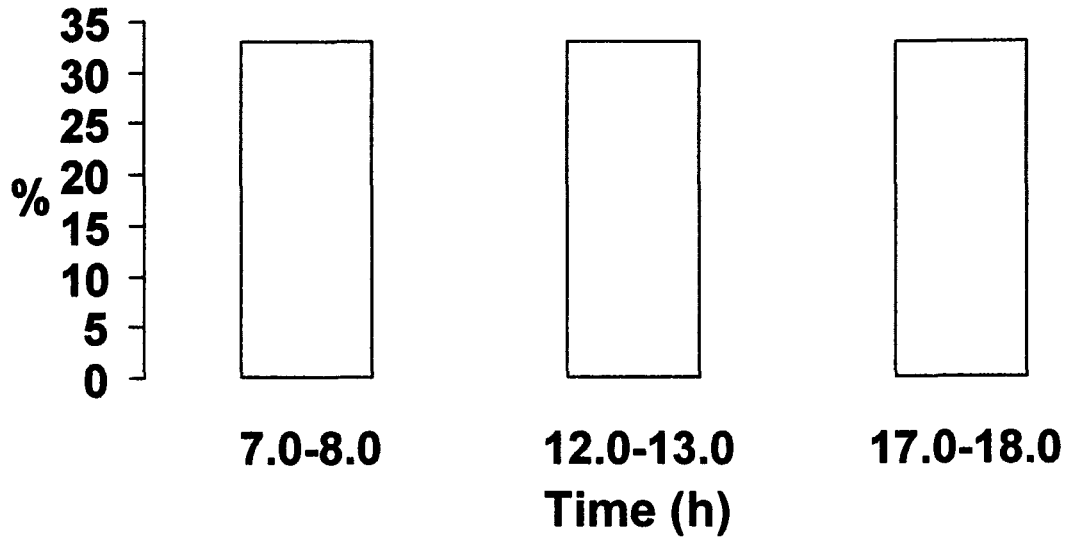
Protocol A

Protocol B
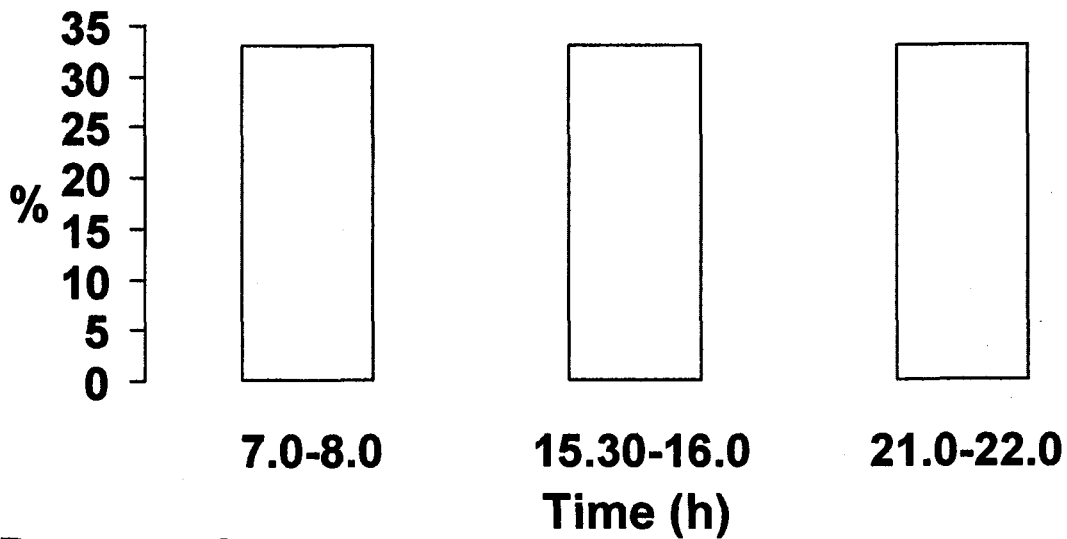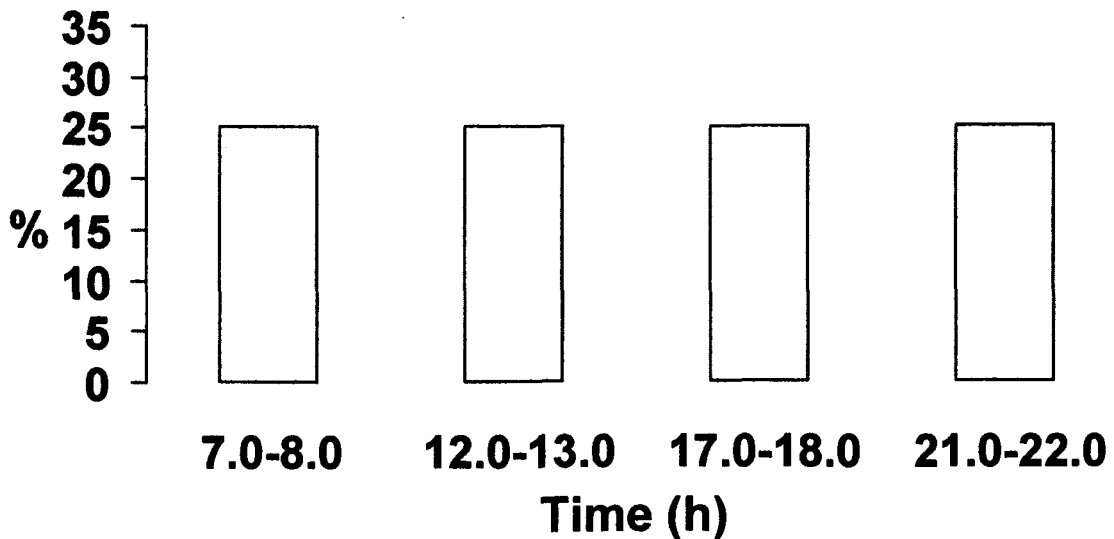
Protocol C

Protocol A : one third of the protein substitute administered in three doses over a 10 hour period, with doses taken between 7.00−8.00 h, 12.00−13.00h, and 17.00−18.00h.

Protocol B: one third of the protein substitute administered in three doses over a 14 hour period, with a dose between 7.00−8.00h, 15.00−16.00h and 21.00−22.00h.

Protocol C: one quarter of the protein substitute administered in four doses over a 14-hour period, with a dose between 7.00−8.00h, 12.00−13.00h, 17.00−18.00h and 21.00−22.00h.

There was no washout time in between the protocols. The randomisation order was generated in blocks of four. Each subject's randomisation order was retained in a sealed envelope until after the subjects or carers had signed the consent form.

Assessment of plasma phenylalanine concentrations

Subjects or their parents took 4 hourly heel, finger or thumb skin puncture blood specimens on day 6 and day 7 of each study period at the following times: 4.00 h, 8.00h, 12.00h, 16.00h, 20.00h and 24.00h. Plasma specimens were repeated for 2 days to test reproducibility at different time points. Blood collection technique had been taught and competence assessed by a specialist nurse. After each study period, blood specimens were collected from home and taken to the laboratory within 8 hours of collection of the final specimen. All blood specimens were centrifuged immediately upon receipt and the resulting plasma samples stored at -20°C until analysed. Plasma phenylalanine concentrations were measured using HPLC as described previously. Subjects maintained their usual activities

throughout the study. All children were free of intercurrent infections at the time of the
study.

Assessment of phenylalanine, protein substitute and energy intake

All subjects were requested to take at least one half to one phenylalanine exchange with each
dose of protein substitute. No more than 50% of the natural protein allowance was taken at
any one meal, although the number of mealtime phenylalanine exchanges could vary during
the study provided the daily allowance was not exceeded.

All food and drink was recorded during day *6* and *7* of each study period to i) monitor
adherence with and timing of phenylalanine exchanges and ii) calculate energy, carbohydrate
and fat intake (there is evidence to suggest that carbohydrate may enhance protein utilisation
and fat may slow the rate of amino acid absorption). Food was weighed using Salter scales
(accurate to 5g) about which parents or older subjects had been instructed. Parents recorded
only food and drink actually consumed; any remaining food was reweighed and deducted
from the start weight. Timing and quantity of protein substitute consumed was recorded
prospectively throughout the trial.

Nutritional analysis of food intake was calculated using the Microdiet computer programme
based on *McCance & Widdowsons's* *'The Composition of Foods'* (Holland *et al,* **1991**) with
supplementary analysis data provided by manufacturers and added to the data base as
described in chapter 2. Energy intake was calculated as a percentage of the estimated
average requirement for energy (DH, **1991**).

6.3 Statistical analyses

One-way analysis of variance was used compare differences between the 3 trial periods.
Pearson's Product Moment Coefficient (r) was used to compare change in phenylalanine
concentrations with timing of protein substitute intake. Multivariate analysis of variance
was used to compare plasma phenylalanine results at all time points simultaneously between
protocols. **A** general linear multivariate analysis of variance was used to compare changes in
plasma phenylalanine concentrations between different time intervals and a Chi-square test
to compare differences between the highest and lowest concentrations in the 3 protocols.

6.4 Results

6.4.1 Subjects withdrawn from the study

Fourteen subjects (11 girls; 3 boys) completed the study. One 2 year old girl was withdrawn
because she refused blood tests at night.

6.4.2 Effect of administration of protein substitute protocol on 24 h plasma phenylalanine pattern

Each of the three different study protocols of protein substitute administration produced a
different 24-hour phenylalanine curve or pattern (Fig 6.2 Protocol **A;** Fig 6.3 Protocol B;
Fig 6.4 Protocol C). Figure 6.5 demonstrates that when comparing simultaneously the
means of all plasma phenylalanine concentrations from all time points in each protocol, the
24-hour profile for each was significantly different **(A** v B; **A v** C; B v C p <0.0001)
(Table 6.1).

Table 6.1

Multivariate analysis of variance comparing the means of all plasma phenylalanine concentrations from all time points simultaneously of the three different protocols

Multivariate analysis of variance for 3 protocols				
Criterion	Test Statistic	F	DF	p value
Wilk's	0.03830	11.507	28	<0.0001
Lawley-Hotelling	11.80322	15.344	26	<0.0001
Pillai's	1.47128	8.348	30	<0.0001
Roy's	10.54115			

Table 6.2

Mean change in plasma phenylalanine concentrations between blood sampling times at 4h intervals in each study protocol

Time intervals (hours)	Mean change in phenylalanine concentrations μmol/l			
	Protocol A (SD)	Protocol B (SD)	Protocol C (SD)	p value
4.00-8.00	58 (6.2)	77 (5.7)	84 (6.2)	<0.02
8.00-12.00	-70 (4.6)	-62 (4.2)	-64 (4.6)	NS
12.00-16.00	-46 (7.0)	67 (6.4)	-27 (7.1)	<0.001
16.00-20.00	-19 (7.7)	-62 (7.0)	-13 (7.7)	<0.001
20.00-24.00	19 (8.9)	-29 (8.1)	-34 (8.9)	<0.001

Table 6.2 demonstrates when comparing the changes in plasma phenylalanine concentrations at 4 hourly intervals between the three protocols using a general linear model of analysis of variance there was a significant difference between protocols for all time points except between 8.00 and 12.00 h.

6.4.3 Change between highest and lowest phenylalanine concentrations observed

Figure *6.6* shows a wide difference between highest and lowest phenylalanine concentrations observed in 24 hours for all three study protocols. The mean **(SD)** change in plasma phenylalanine concentrations (μmol/l) were as follows:

Protocol **A:**   136   (38)

Protocol B: 110   (32)

Protocol C: 129   (42)

Protocol B produced significantly less change than the other two protocols. In protocol B, 79% of phenylalanine concentrations changed by 120 μmol/l or less within all the 24 hour time periods studied compared with 35% in protocol **A;** and 48% in protocol *C* ($p < 0.005$) (Fig *6.6)*.

6.4.4 Effect of time since giving dose of protein substitute and change in plasma phenylalanine concentrations

When examining the interval since giving the last dose of protein substitute and change in plasma phenylalanine concentration there appears to be a similar pattern in all 3 protocols (Figs **6.7** - 6.12). When examining all and mean changes in plasma phenylalanine

concentrations in all 3 protocols, there was an initial depression in phenylalanine concentration reaching a peak at 4 hours. This was then followed by a rapid increase in concentrations, which reached a plateau after a further 3 – 4 hours.

6.4.5 Phenylalanine concentrations outside the MRC (l993b) recommended guidelines

Figure 6.13 shows that all three methods of administering protein substitute resulted in more than a quarter of all phenylalanine concentrations being lower than the MRC recommended guidelines (MRC, 1993b), despite 08.00h concentrations being within the target range. In protocol **A, 29%** of all phenylalanine concentrations were below 100μmol/l, **3** 1% in protocol B and *26%* in protocol C. There were no significant differences between the three groups.

Only **5%** of phenylalanine concentrations exceeded the MRC upper limit of 700 μmol/l in protocol C (MRC, 1993b). This was in **2** older subjects who both had a 08.00h concentration just above the accepted range.

6.4.6 Within subject reproducibility of plasma phenylalanine concentrations on day 6 and day 7 of each study protocol

There was good within subject reproducibility for the majority of the subjects between phenylalanine concentrations taken at the same times on the consecutive days of each protocol. For 90% of the individuals the correlation coefficient *(r)* was >0.6 for the three study protocols.

Table 6.3

Distribution of energy intake

Time Zone	% energy intake (SD) Protocol			% fat intake (SD) Protocol			% carbohydrate intake (SD) Protocol		
	A	B	C	A	B	C	A	B	C
8.00 – 11.59	28 (5.1)	26 (5.1)	24 (5.1)	21 (7.2)	20 (10.7)	23 (12)	25 (10)	25 (6)	22 (6)
12.00 – 15.59	34 (6.4)	34 (9.6)	35 (5.7)	34 (22)	37 (13)	35 (13)	35 (7)	33 (9)	34 (6)
16.00 – 19.59	33 (7.7) *	23 (7.8) *	31 (6.0)	36 (16)	30 (13)	31 (12)	31 (8)	25 (10)	31 (7)
20.00 – 24.00	7 (8.8) **	17 (8.9) **	12 (6.4)	7 (10)	8 (10)	6 (10)	10 (12)	17 (10)	18 (10)

*   A versus B p <0.05

* *   A versus B p <0.05

6.4.7 Protein and energy intake

There was no significant difference in overall energy intake between the three protocols.
The mean daily energy intake as a percentage of **EAR** (DH, 1991) for protocols **A,** B and C
was 94% (SD **27%),** 96% (SD 19%) and **97%** (SD 20%) respectively. Energy intake was
also calculated every four hours to coincide with the blood sampling times. Overall, there
was no difference in energy intake before 16.00h between the three protocols, but there was
less energy consumed between 16.00 to 20.00h in protocol B ($p < 0.01$) when no protein
substitute was administered and more after 20.00h when 33% of protein substitute was taken
($p < 0.05$). **This** was due exclusively to the carbohydrate added to the protein substitute and
energy supplied by amino acids (Table 6.3).

The total protein intake was similar in the three protocols (mean % protein intake of the
RNI: protocol **A:** 253% (SD 53%); B: 261% (SD 55%); and C: 262% **(SD 54%)).** There was
also no significant difference in natural protein intake between the three protocols or within
the same four-hour time periods for which blood specimens were taken (Table 6.4).

Table 6.4

Distribution of protein intake

Time Zone	Distribution of total protein intake % (SD)			Distribution of total natural protein intake g (SD)			Distribution of 'official' phenylalanine exchanges g (SD)		
	Protocol			Protocol			Protocol		
	A	B	C	A	B	C	A	B	C
8.00 – 11.59	32 (2.1) *	31 (2.4) *	25 (1.2) *	2.5 (1.3)	2.0 (0.6)	2.5 (1.5)	1.9 (0.8)	1.5 (0.7)	2.2 (1.3)
12.00 – 15.59	33 (2.3)	30 (11.7)	28 (3.2)	3.8 (2.4)	4.3 (3.0)	4.3 (2.9)	2.3 (2.0)	2.8 (2.4)	2.6 (2.1)
16.00 – 19.59	34 (2.3) * *	10 (9.8) * *	28 (3.7) * *	3.4 (2.6)	4.0 (2.6)	3.8 (2.0)	2.5 (1.7)	2.7 (1.7)	2.1 (1.1)
20.00 – 24.00	2 (3.1) * * *	30 (4.4) * * *	22 (4.6) * * *	0.9 (1.7)	0.6 (0.7)	0.7 (0.9)	0.5 (0.9)	0.4 (0.6)	0.5 (0.8)

* A versus C $p<0.001$ * * A versus B $p<0.001$ * * * A versus B $p<0.001$

* B versus C $p<0.001$ * * A versus C $p<0.05$ * * * A versus C $p<0.01$

       * * * B versus C $p<0.05$

6.5 Summary

This study demonstrates that manipulation of the timing and dosage of protein substitute in PKU changes the plasma phenylalanine pattern over 24-hours. However, administration of protein substitute over extended daytime hours and in smaller doses does not lead to improved control of phenylalanine concentrations. With all 3 methods of administering protein substitute, the difference between the highest and lowest concentrations still exceeded $100\mu mol/l$. However, this study does show that administration of $25-33\%$ of the daily protein substitute allowance at any one time causes a decrease in phenylalanine concentrations. This change seems to persist for approximately four hours. If protein substitute was not given for more than 4 hours, plasma phenylalanine concentrations rose quickly. Therefore, adjusting the timing of protein substitute during daytime hours does not improve 24-hour plasma phenylalanine profile.

Fig 6.2   24h plasma phenylalanine profile for all subjects for Protocol A (when 33% protein substitute administered between 7.00-8.00h, 12.00-13.00h and 17.00-18.00h)
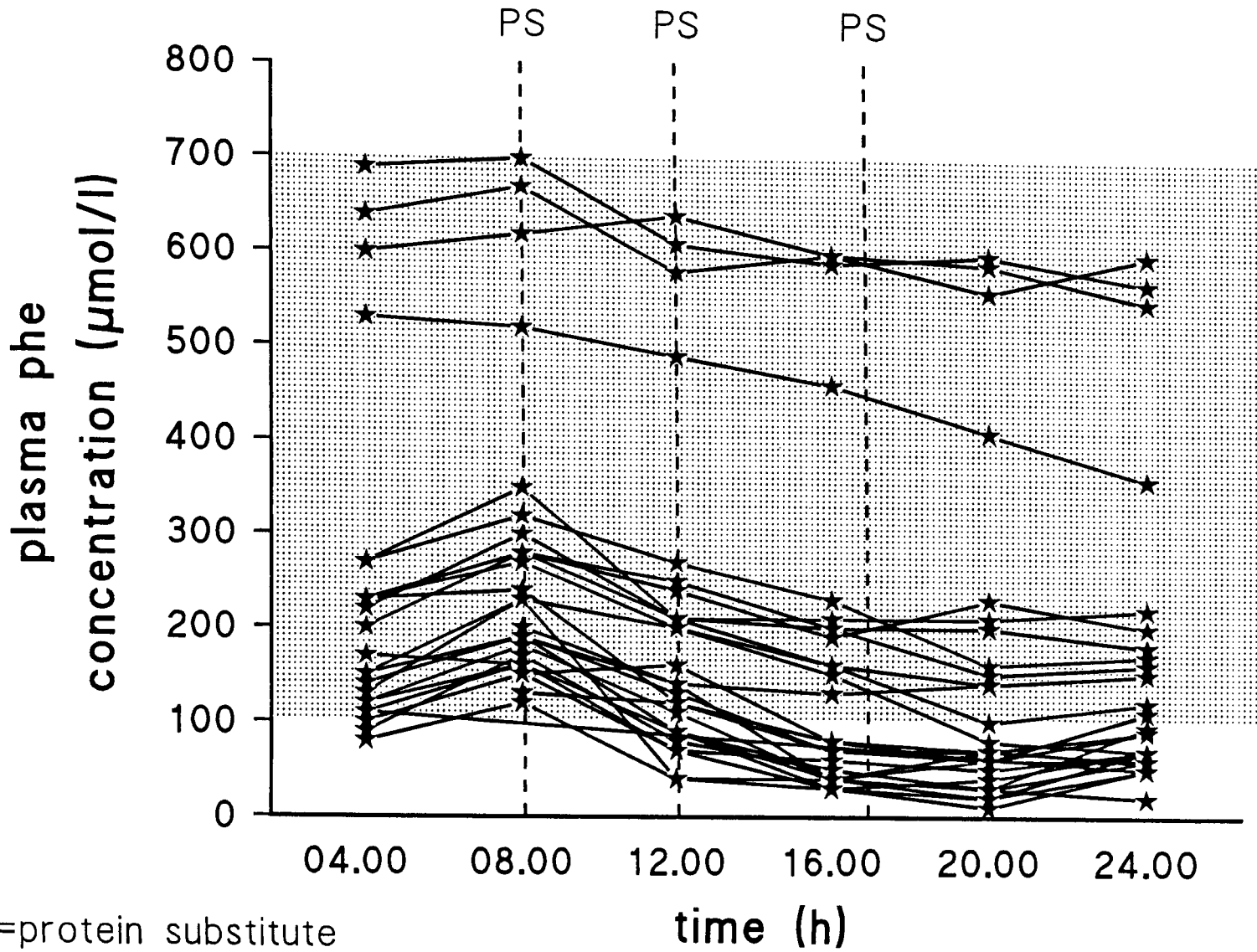
PS=protein substitute

Fig 6.3 **24h plasma phenylalanine profile for all subjects for Protocol B (when 33% protein substitute administered between 7.00-8.00h, 15.30-16.00h and 21.00-22.00h)**
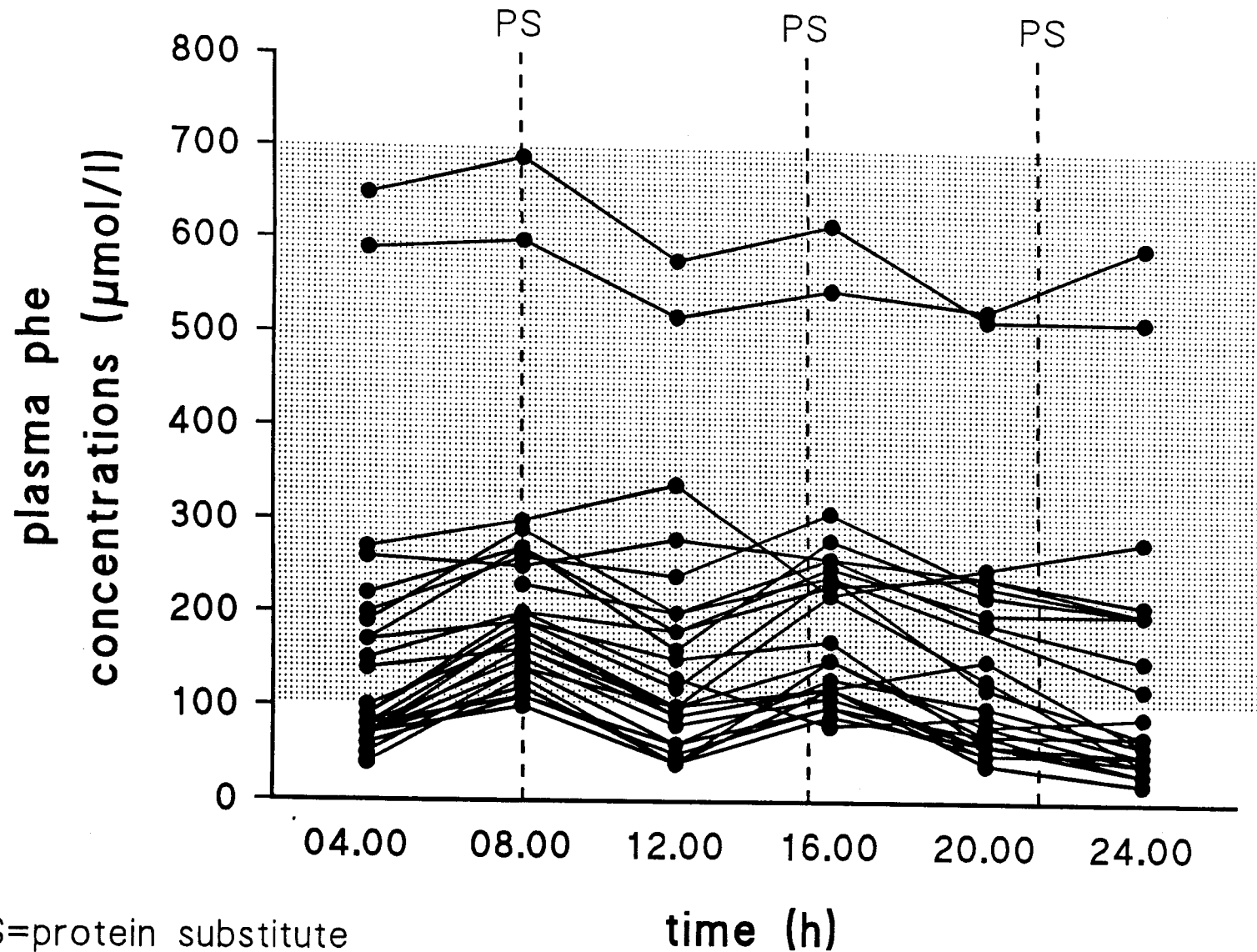
PS=protein substitute

Fig 6.4   24h plasma phenylalanine profile for all subjects for Protocol C (when 25% protein substitute administered between 7.00-8.00h, 12.00-13.00h, 17.00-18.00h and 21.00-22.00h)

PS=protein substitute
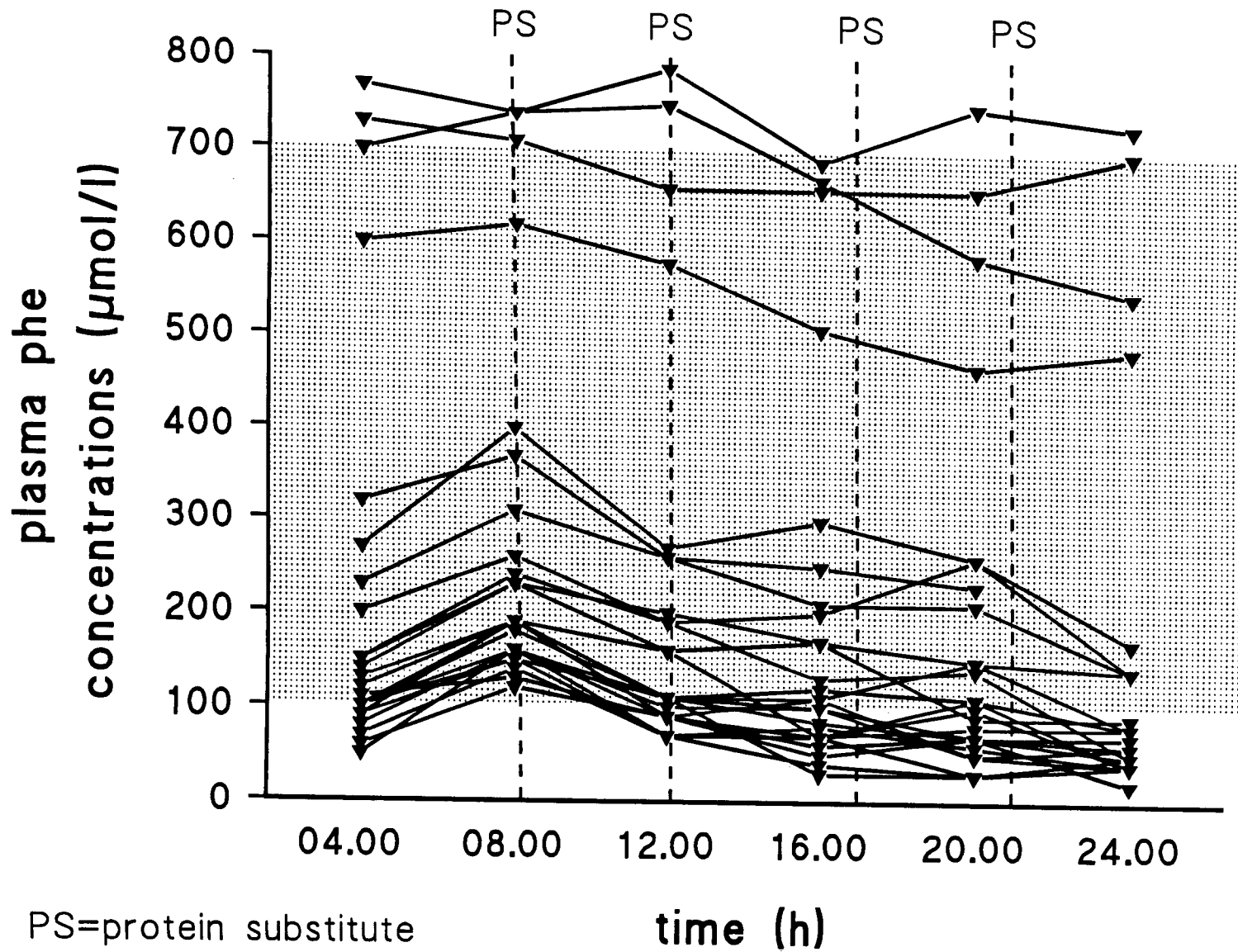
Fig 6.5 **Comparison of mean 24h plasma profiles or curves from Protocol A, B and C (T=SEM)**
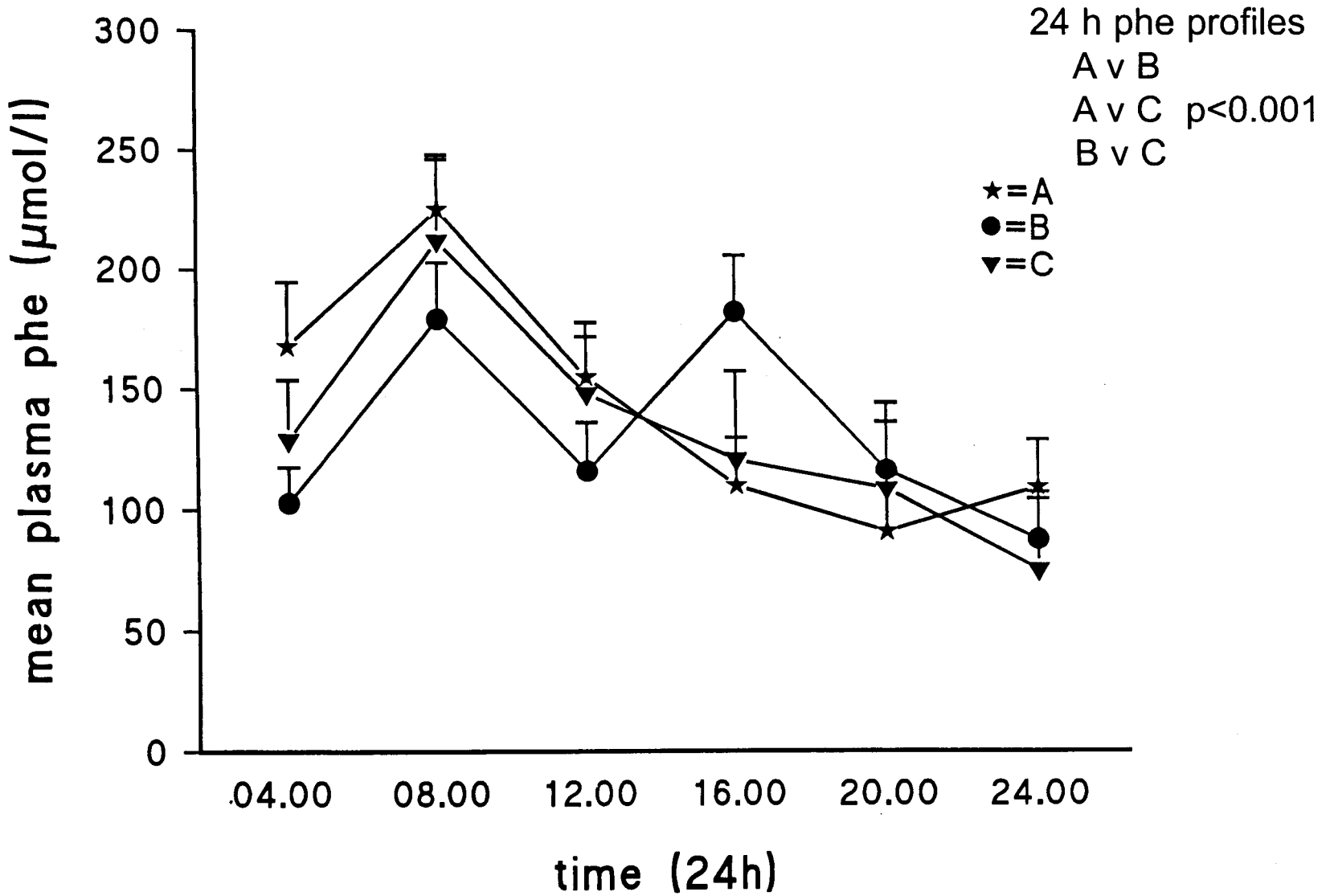
Fig 6.6 The change between highest and lowest plasma phenylalanine concentrations over 24 hours for all subjects in Protocol A, B and C

Fig 6.7   The effect of length of time from taking last dose of protein substitute on change in all plasma phenylalanine concentrations from protocol A
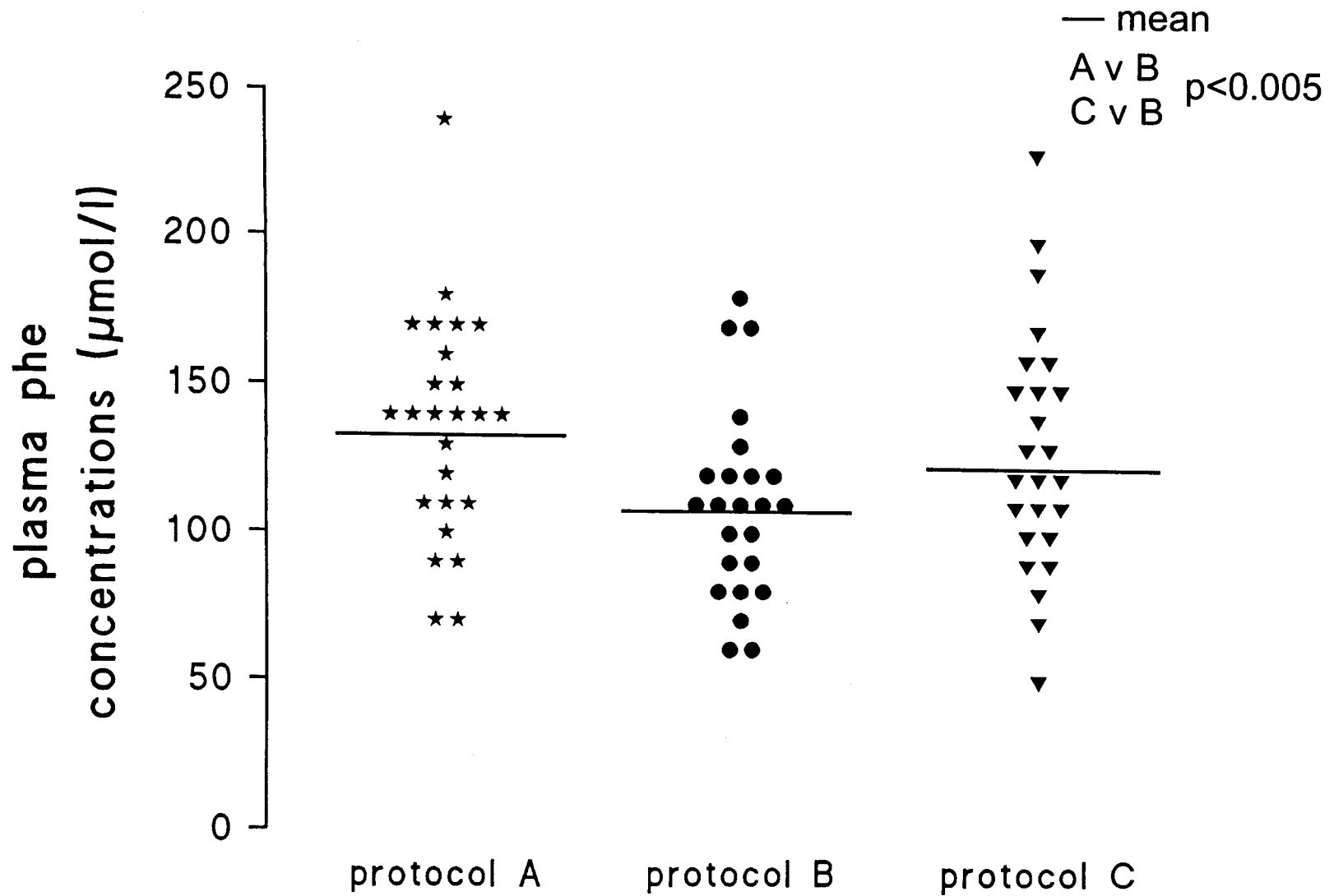
time (h) from taking dose of protein substitute
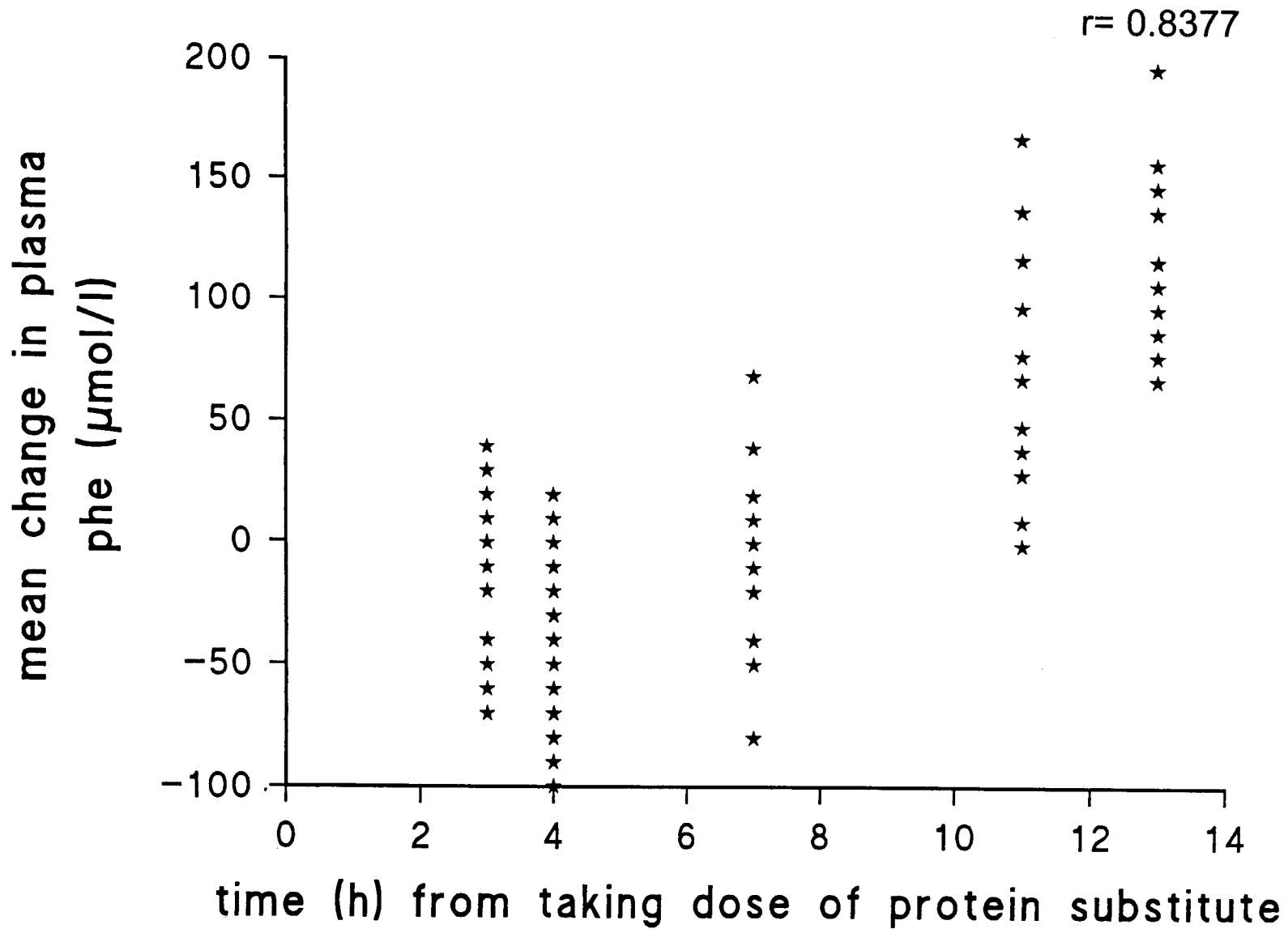
Fig 6.8 The effect of length of time from taking last dose of protein substitute on change in mean plasma phenylalanine concentrations from protocol A (I=SEM)
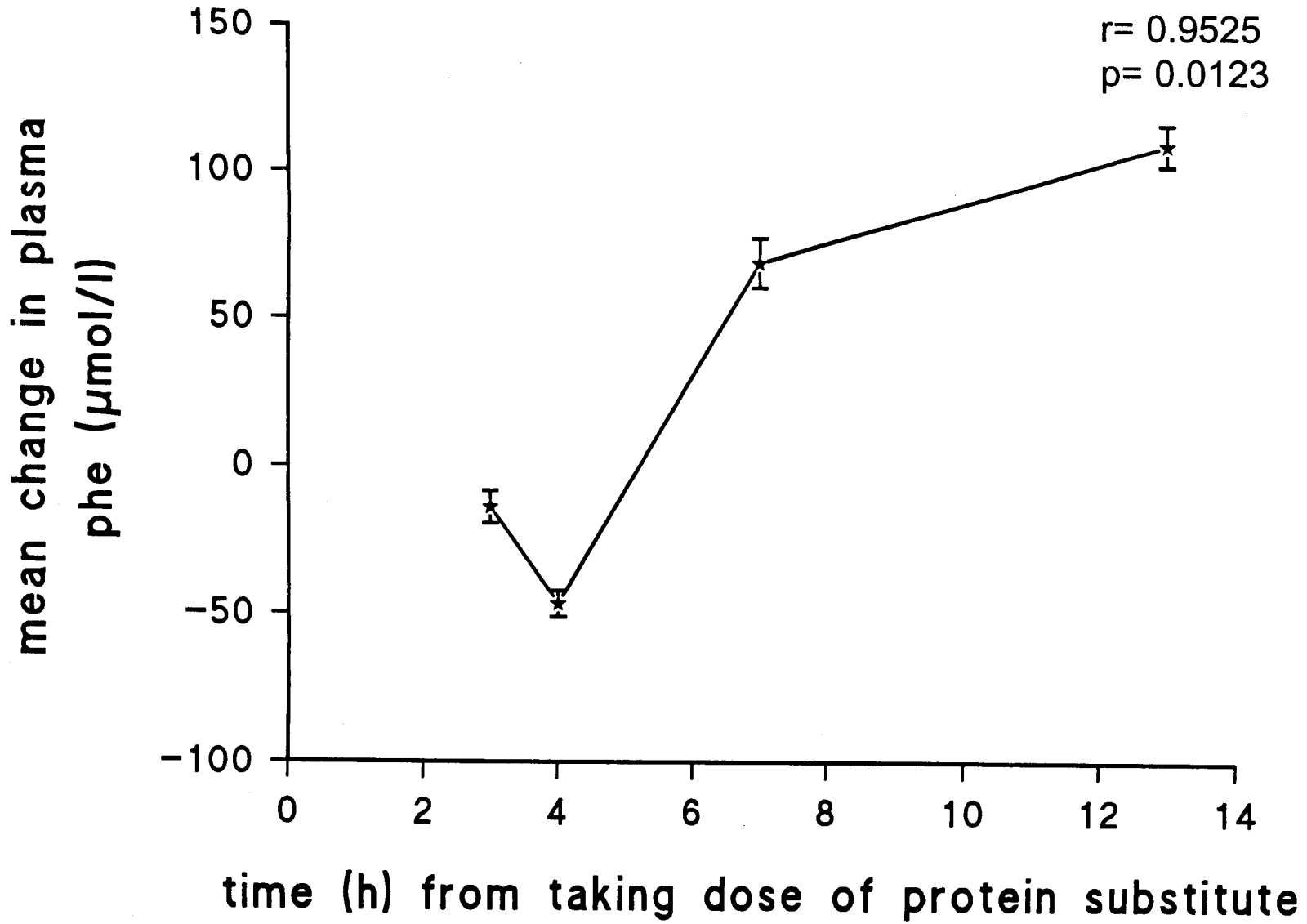
r= 0.9525
p= 0.0123

(y-axis) mean change in plasma phe (μmol/l)

(x-axis) time (h) from taking dose of protein substitute

Fig 6.9 **The effect of length of time from taking last dose of protein substitute on change in all plasma phenylalanine concentrations from protocol B**

r=0.6995

mean change in plasma phe (μmol/l)

time (h) from taking dose of protein substitute
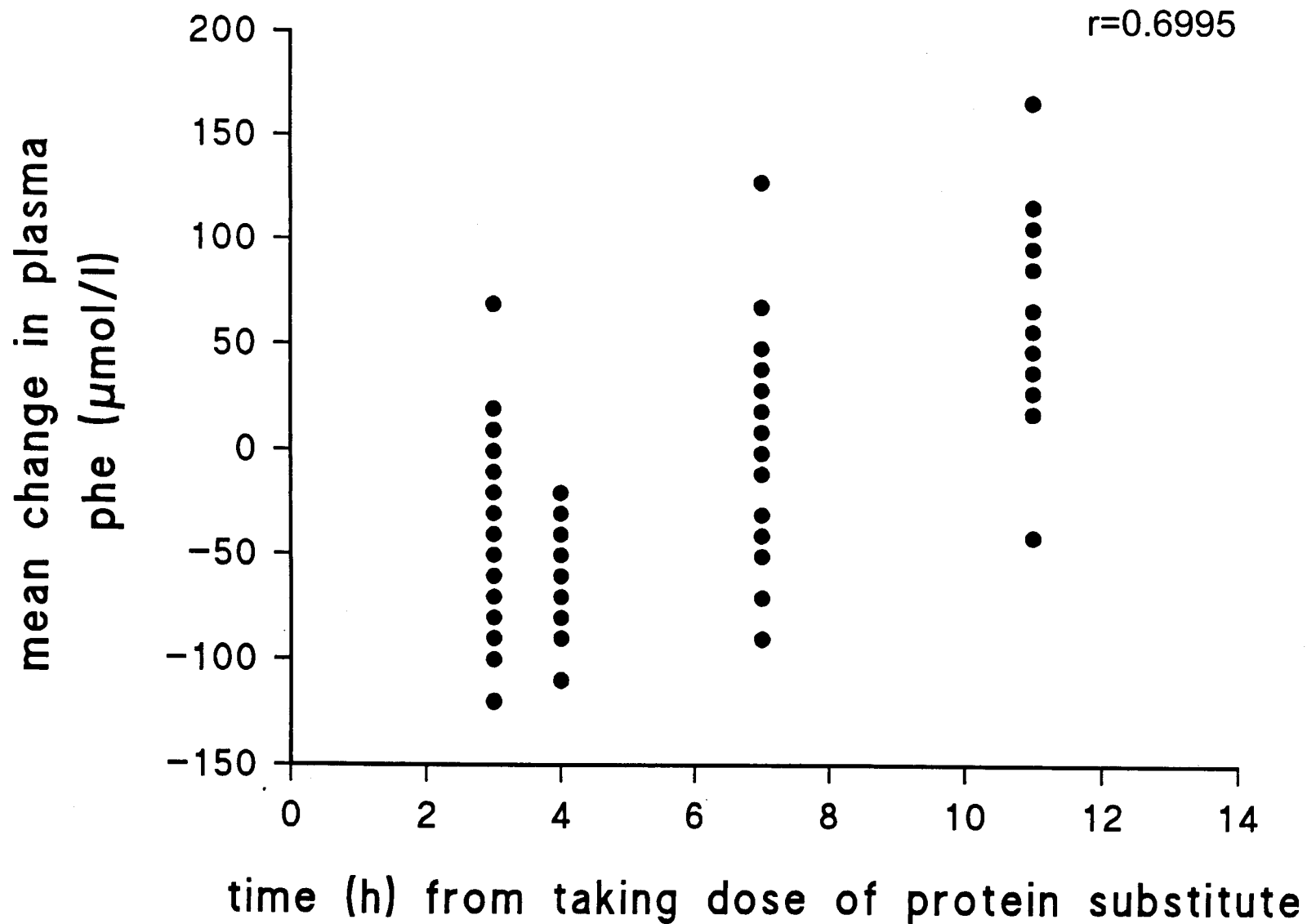
Fig 6.10 The effect of length of time from taking last dose of protein substitute on change in mean plasma phenylalanine concentrations from protocol B (I=SEM)

r=0.9399
p=0.0601

mean change in plasma phe (μmol/l)

time (h) from taking dose of protein substitute
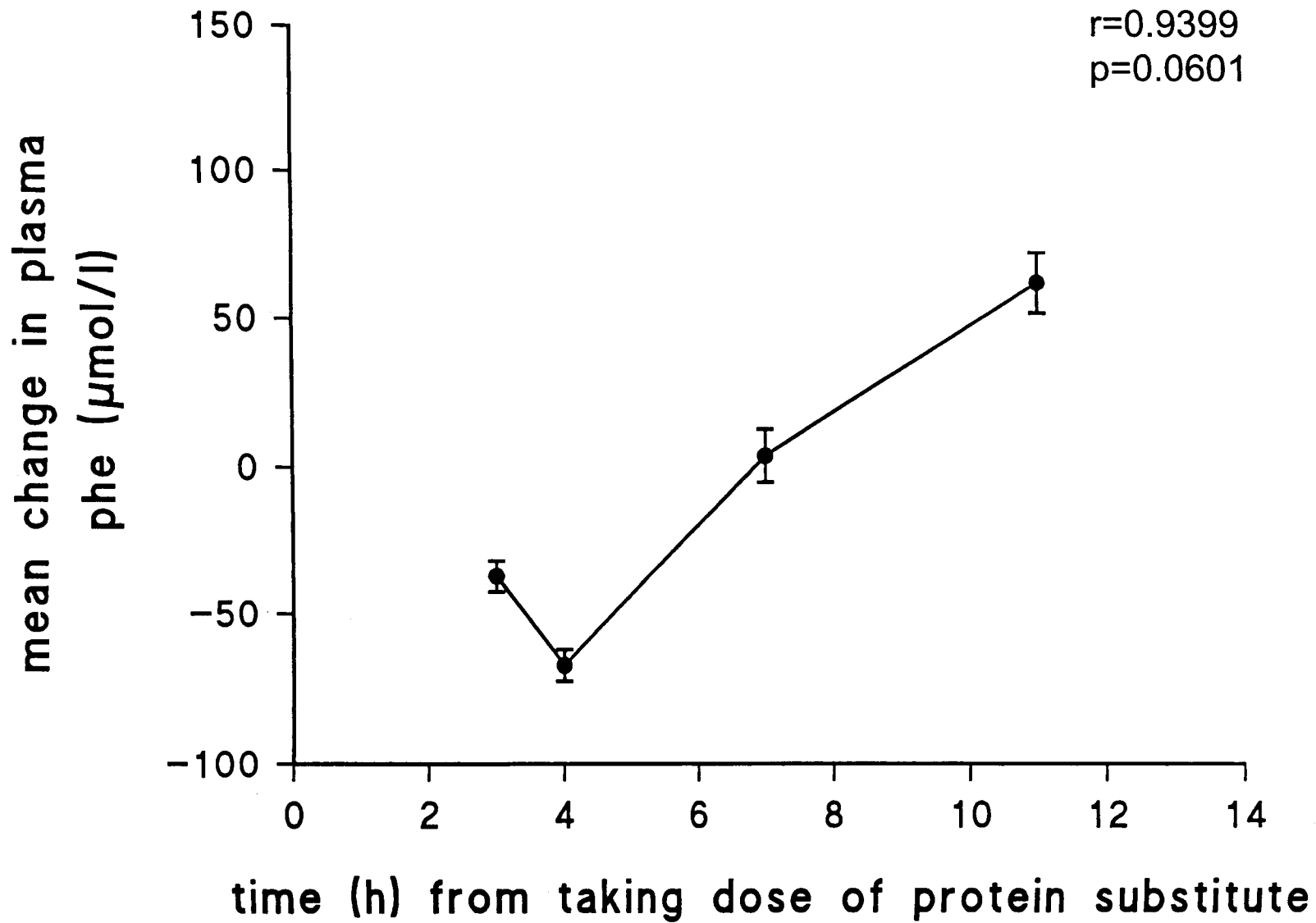
Fig 6.11 **The effect of length of time from taking last dose of protein substitute on change in mean plasma phenylalanine concentrations from protocol C**
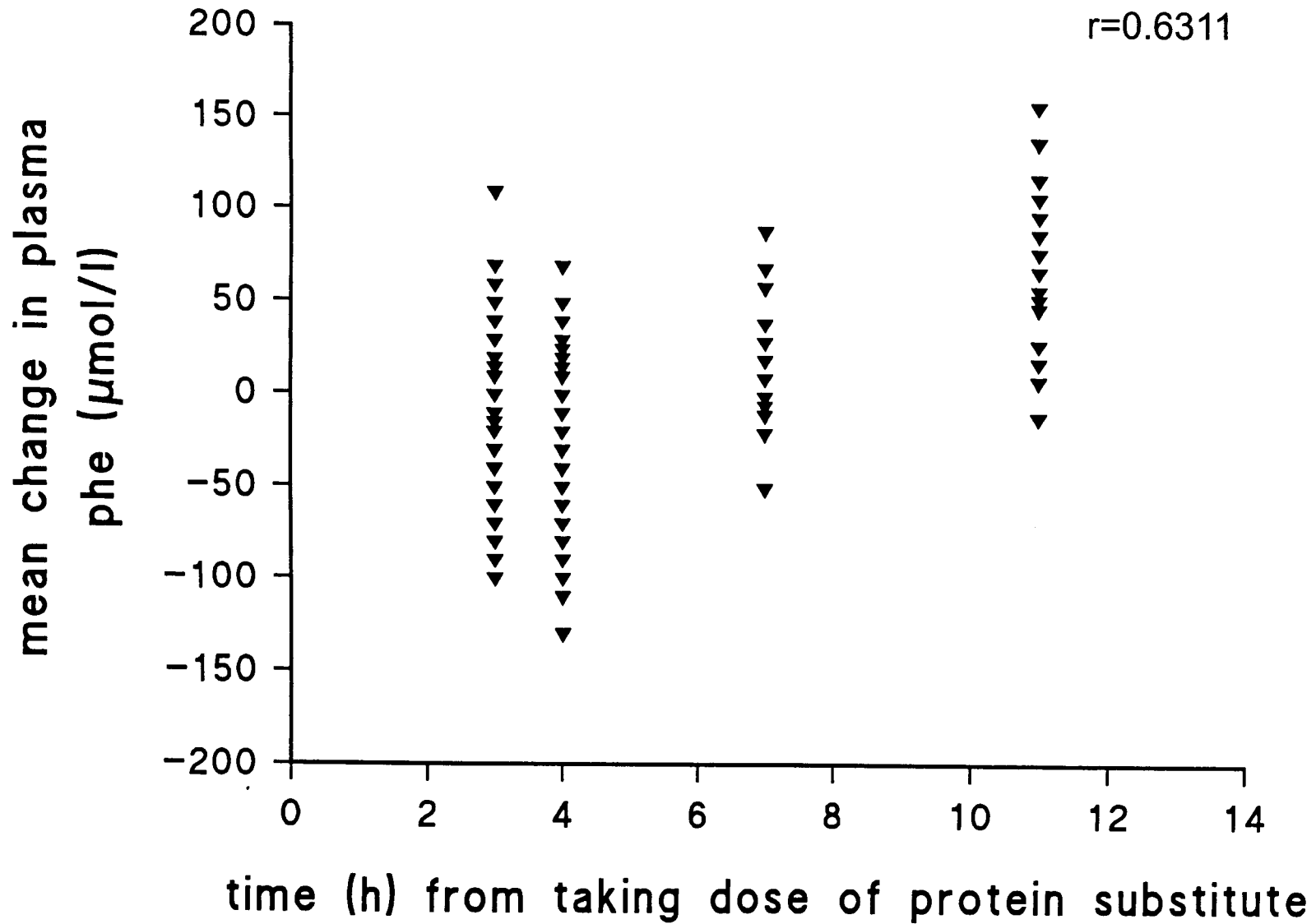
Fig 6.12 The effect of length of time from taking last dose of protein substitute on change in mean plasma phenylalanine concentrations from protocol C (I=SEM)
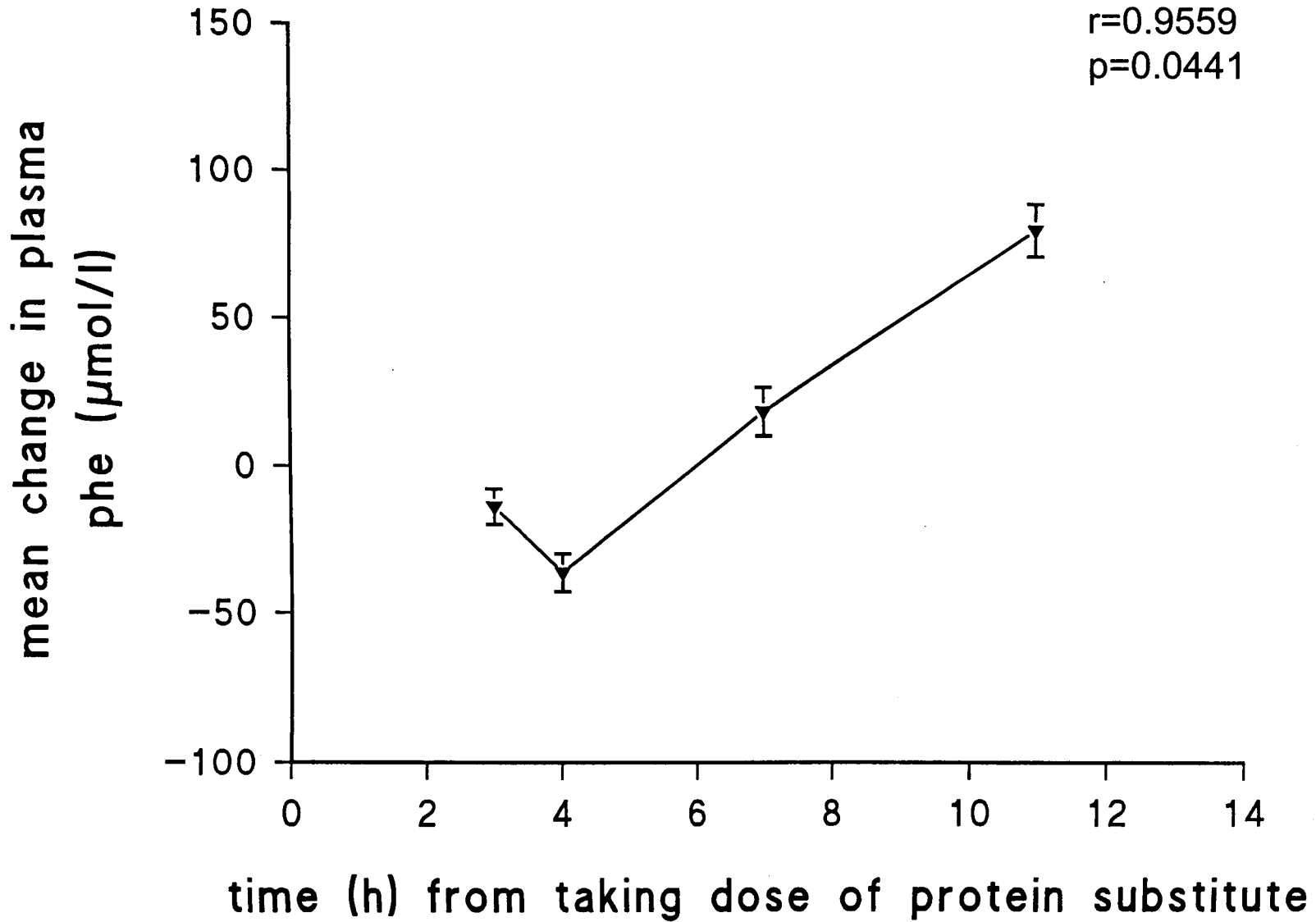
Fig 6.13 A summary of all 24h plasma phenylalanine concentrations from each of the different time points for all subjects for protocol A, B and C

— median

protocol A   protocol B   protocol C
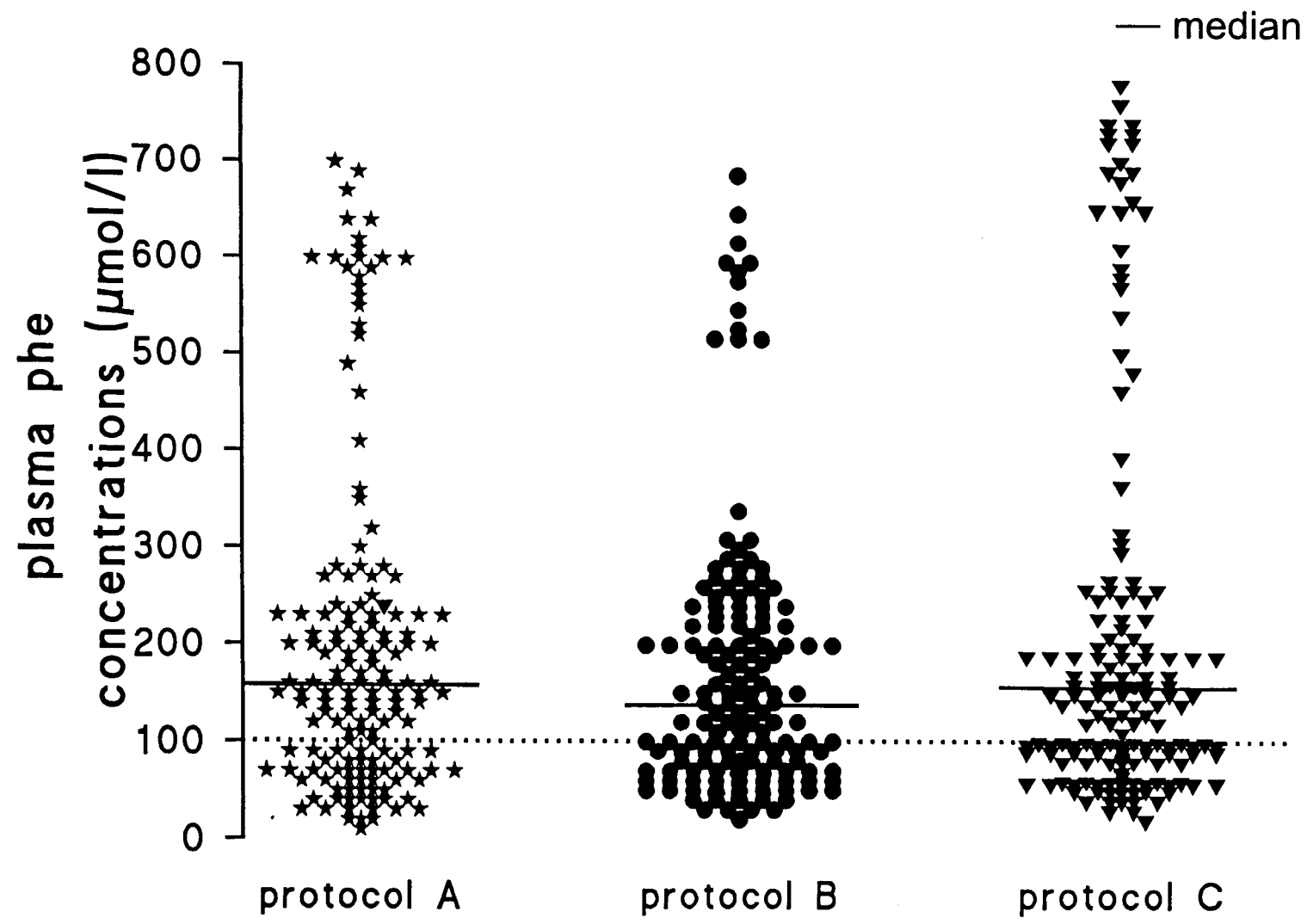
Chapter 6

Part B

The effects of frequent administration of protein substitute on plasma phenylalanine concentrations

6.6 Introduction

In chapter **6A,** it was demonstrated that protein substitute in three to four daytime doses caused non-physiological variability in plasma phenylalanine concentrations in PKU. Giving protein substitute depressed phenylalanine concentrations and there is some evidence to suggest that the effect is related to the quantity and timing of protein substitute intake.

It was hypothesised that plasma phenylalanine variability will be significantly reduced by giving protein substitute in small equal doses, every four hours both day and night. Therefore, in a small, randomised, cross-over study, the effect of administering protein substitute every four hours day and night on plasma phenylalanine was compared with two different protocols of giving protein substitute in three equal doses during the day.

6.7 Subjects and methods

6.7.1 Subjects

Three patients (all Caucasian) with well-controlled classical PKU were recruited. The inclusion criteria were the same as described in the previous study. Two girls and one boy, aged **7, 8** and 11 years fulfilled these criteria and agreed to enter the study. Five, **6** and 7.5 fifty-milligram phenylalanine exchanges were allocated to each child respectively. The two children under 10 years took 2.0g/kg/day of protein substitute, and the patient over 11 years 1.5g/kg/day. They used their usual brand of protein substitute. The two girls took XP Maxamaid (Scientific Hospital Supplies), supplemented with Aminogran Food Supplement (UCB Pharma) and the boy took Phlexy 10 Drink Mix (Scientific Hospital Supplies) to achieve target protein requirements.

Fig 6.14   Study design: Percentage dosage and time of administering protein substitute (Protocol A, Protocol B, Protocol C)

Protocol A

%

Time (h): 7.0-8.0   12.0-13.0   17.0-18.0

Protocol B

%

Time (h): 7.0-8.0   15.30-16.0   21.0-22.0

Protocol C

%

Time (h): 8.0   12.0   16.0   20.0   24.0   4.0
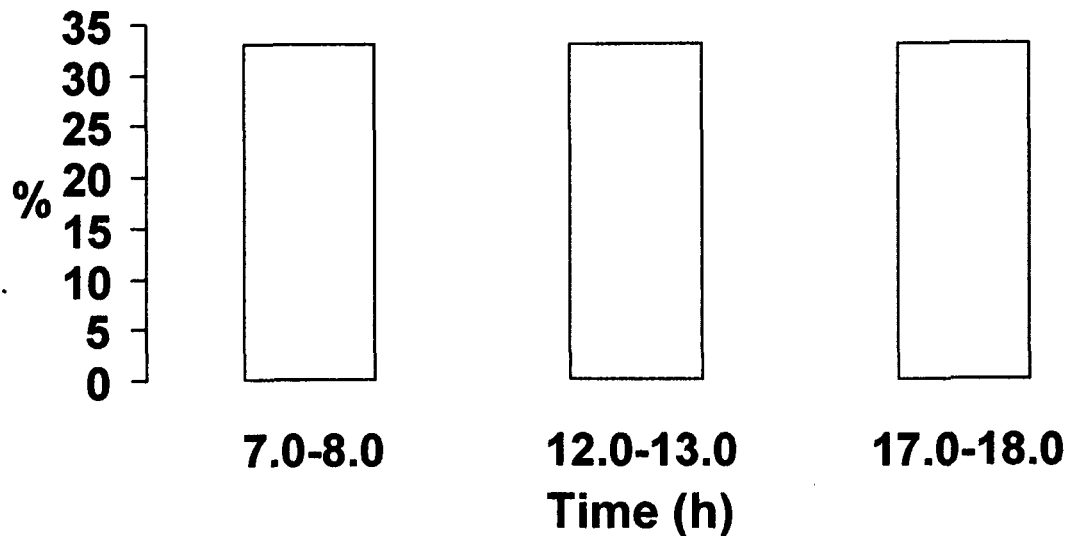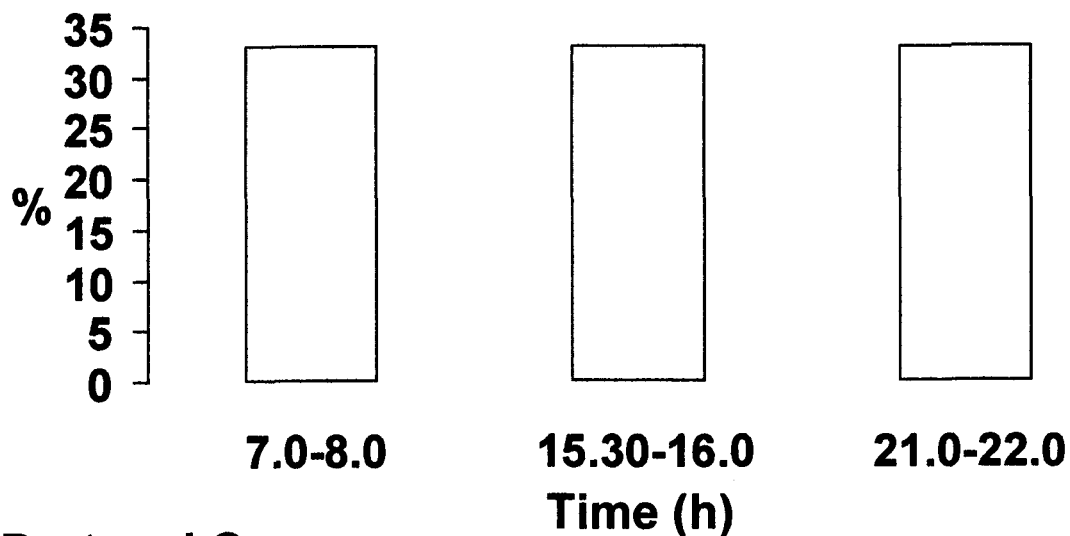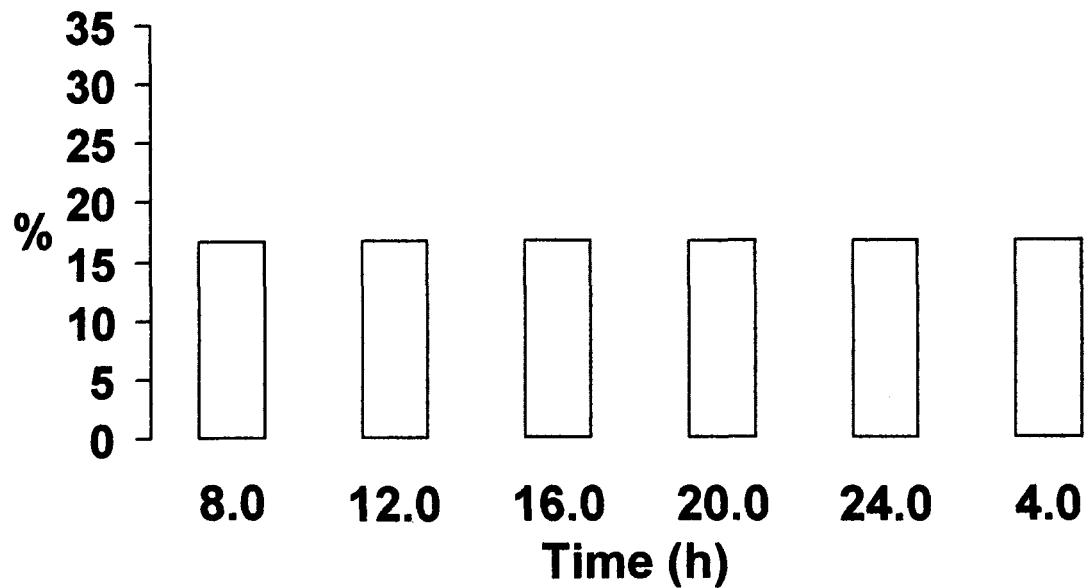
6.7.2 Study design

The study design was similar to the previous protein substitute timing study, with the protein substitute administered in 3 different ways over separate protocols to each subject. Protocol **A** and B were exactly the same as before. However, in protocol C, patients took their protein substitute in 6 equal doses at 8.00h, 12.00h, 16.00h, 20,00h, 24.00h and 4.00h for 3 consecutive days only (Fig 6.14). Parents took 4 hourly finger or thumb skin puncture blood specimens on the last 2 days of each study protocol at the following times: 4.00h, 8.00h, 12.00h, 16.00h, 20.00h and 24.00h. They were analysed as before.

The patients were requested not to take a phenylalanine exchange with every dose of protein substitute in protocol C, due to the impracticalities of expecting children to eat as well as take protein substitute during the night. However, it was requested phenylalanine exchanges were taken with all protein substitute doses in protocol A and B.

All food and drink consumed was recorded as before on the last 2 days of each protocol.

6.8 Statistical analyses

No statistical analyses were performed as only **3** subjects were studied.

6.9 Results

6.9.1 Effect of administration of protein substitute protocol on 24h plasma phenylalanine pattern

Each of the three different study protocols of protein substitute administration produced a different 24-hour phenylalanine curve or pattern. The pattern for protocol A (Fig 6.15) and

protocol B (Fig 6.16) was similar to the ones observed in the previous study (chapter **6A**).
However, the pattern observed in protocol **C** (Fig 6.17) was very different. There was little change in plasma phenylalanine concentrations between different time points, and almost a straight line was produced over 24 hours. The majority of phenylalanine concentrations were below 100 μmol/l.

The mean (range) of plasma phenylalanine concentrations for all subjects at 4 hourly intervals, from the three different protocols, is given in Table 6.5.

Table 6.5

Mean plasma phenylalanine concentrations for 4 hourly intervals for three different protocols of administering protein substitute

Time	Mean plasma phenylalanine concentrations μmol/l (range)		
	Study protocol A	Study protocol B	Study protocol C
4.00 h	265 (160 – 360)	213 (50 – 370)	78 (60 – 110)
8.00 h	322 (240 – 410)	268 (80 – 430)	78 (60 – 100)
12.00 h	252 (160 – 340)	208 (30 –380)	90 (60 – 140)
16.00 h	205 (100 – 300)	252 (40 –410)	67 (50 – 90)
20.00 h	173 (30 – 260)	185 (30 – 360)	60 (40 – 80)
24.00 h	175 (40 – 280)	222 (40 – 410)	72 (40 – 90)

6.9.2 Change between highest and lowest phenylalanine concentrations observed

Figure 6.18 indicates the change between highest and lowest observations was much lower for protocol C, than protocol **A** and *C*.

The mean (SD) difference in plasma phenylalanine concentrations (μmol/l) were as follows:

Protocol **A**:   160   (100 – 240)

Protocol B: 90   (50 – 170)

Protocol C: 43   (20 – 80)

Mean change in blood phenylalanine concentrations between 4 hourly blood specimens did not exceed –23 μmol/l (range 18 to –23 μmol/l) in protocol *C* compared with a maximum mean change of -60 μmol/l (range -60 to *55* μmol/l) in protocol B and 115 μmol/l (range -65 to 115 μmol/l) in protocol **A.**

6.9.3 Energy and protein intake

The mean energy intake compared with the **EAR** (DH 1991) was 103% (range 66–131%) in protocol **A,** 92% (range 78–115%) in protocol **B,** and 109% (81–126%) in protocol C (Table 6.6). **A** small quantity of energy supplied by the amino acids and carbohydrate added to the protein substitute was taken throughout the night in protocol *C,* but was much less than the energy consumed during the day. The total protein intake (from protein substitute and natural protein) was much higher than RNI (DH, 1991) in all three protocols (protocol **A:** mean 246% (range 167–300%); protocol B: mean 212% (range 161–252%); protocol C: mean 236% (range 170–304%). There was a large discrepancy between the

allocated phenylalanine (protein) exchanges and actual natural protein intake. Although the mean allocated phenylalanine exchanges (assuming 50 mg phenylalanine is equivalent to 1g natural protein) was 6.2 daily, the mean natural protein consumed in protocol A was 11.4g (range 10–13g) daily. In protocol B, it was 9.7g (range 7.7-12.4g) daily and, in protocol C, it was 12.1g (range 8.4-15g) daily. Natural protein was not taken with every dose of protein substitute; and it was not equally distributed with meals. Indeed, over 50% of the daily natural protein were consumed in four out of nine meals in both protocols B and *C* (Table 6.7). However, this did not appear to adversely affect blood phenylalanine concentrations.

6.10 Summary

The repeated administration of protein substitute every four hours during the day and night leads to marked stabilisation of plasma phenylalanine concentrations in PKU. In fact, it produced a profile similar to the physiological pattern seen in non-PKU humans. It reduced mean daily variability between highest and lowest phenylalanine concentrations to less than 50 μmol/l. In the few patients studied, it suppressed all phenylalanine concentrations, and they ran just below the lower MRC recommended limit for PKU (MRC, 1993b). However, concentrations remained stable and no concentrations fell below the lower normal accepted range for phenylalanine. Therefore, continual administration of protein substitute appears to be an effective tool in reducing phenylalanine variability in PKU.

Table 6.6

Distribution of energy, fat and carbohydrate intake

Time	Energy % EAR (range)			Fat % energy (range)			Carbohydrate % energy (range)		
	Study protocol A	Study protocol B	Study protocol C	Study protocol A	Study protocol B	Study protocol C	Study protocol A	Study protocol B	Study protocol C
8.00 – 11.59 h	21 (12 – 32)	20 (13 – 27)	26 (20 – 30)	15 (1 – 40)	16 (1 – 38)	26 (16 – 38)	22 (14 – 29)	22 (15 – 28)	27 (22 – 37)
12.00 – 15.59 h	37 (28 – 51)	38 (32 – 47)	29 (22 – 43)	35 (18 – 54)	43 (29 – 55)	35 (24 – 61)	38 (25 – 55)	37 (32 – 48)	29 (22 – 40)
16.00 – 19.59 h	31 (23 – 41)	17 (2 – 40)	31 (21 – 43)	41 (20.38)	33 (16 – 60)	36 (11 – 50)	34 (21 – 56)	22 (7 – 42)	32 (18 – 44)
20.00 – 23.59 h	7 (0 – 16)	18 (11 – 42)	4 (4 – 6)	10 (0 – 41)	9 (1 – 36)	0	7 (0 – 15)	19 (10 – 45)	4 (2 – 6)
24.00 – 3.59 h	0	0	4 (0)	0	0	0	0	0	4 (2 – 6)
4.00 – 7.59 h	0	0	4 (0)	0	0	0	0	0	4 (2 – 6)

Table 6.7

Distribution of protein intake

Time	Mean % total protein intake (range)			Mean natural protein g (range)			Mean % natural protein intake (range)		
	Study protocol A	Study protocol B	Study protocol C	Study protocol A	Study protocol B	Study protocol C	Study protocol A	Study protocol B	Study protocol C
8.00 – 11.59 h	27 (12 – 35)	26 (16 – 32)	22 (18 – 28)	1.7 (0 – 3.1)	0.7 (0 – 2)	2.9 (0.6 – 4.1)	17 (0 – 31)	8 (0 – 22)	23 (5 – 33)
12.00 – 15.59 h	33 (29 – 36)	35 (32 – 41)	18 (16 – 20)	4.2 (2.4 – 6.5)	4.3 (1.6 – 4.1)	2.4 (1.6 – 4.1)	37 (23 – 49)	41 (21 – 79)	20 (12 – 36)
16.00 – 19.59 h	30 (21 – 38)	4 (2 – 7)	21 (16 – 27)	2.8 (0.9 – 6.9)	3.2 (0.9 – 6.9)	7.3 (6.0 – 9.2)	30 (8 – 62)	36 (21 – 70)	55 (49 –64)
20.00 – 23.59 h	3 (0 – 8)	28 (21 – 38)	13 (12 – 14)	3.1 (0 – 5.1)	3.9 (0 – 10.3)	0	18 (0 – 45)	12 (0 – 68)	0.7 (0 – 4)
24.00 – 3.59 h	0	0	14 (13 – 15)	0	0	0	0	0	0
4.00 – 7.59 h	0	0	14 (13 – 15)	0	0	0	0	0	0

Fig 6.15   24h plasma phenylalanine profile for all subjects for Protocol A (when 33% protein substitute administered between 7.00-8.00h, 12.00-13.00h, 17.00-18.00h)

PS=protein substitute
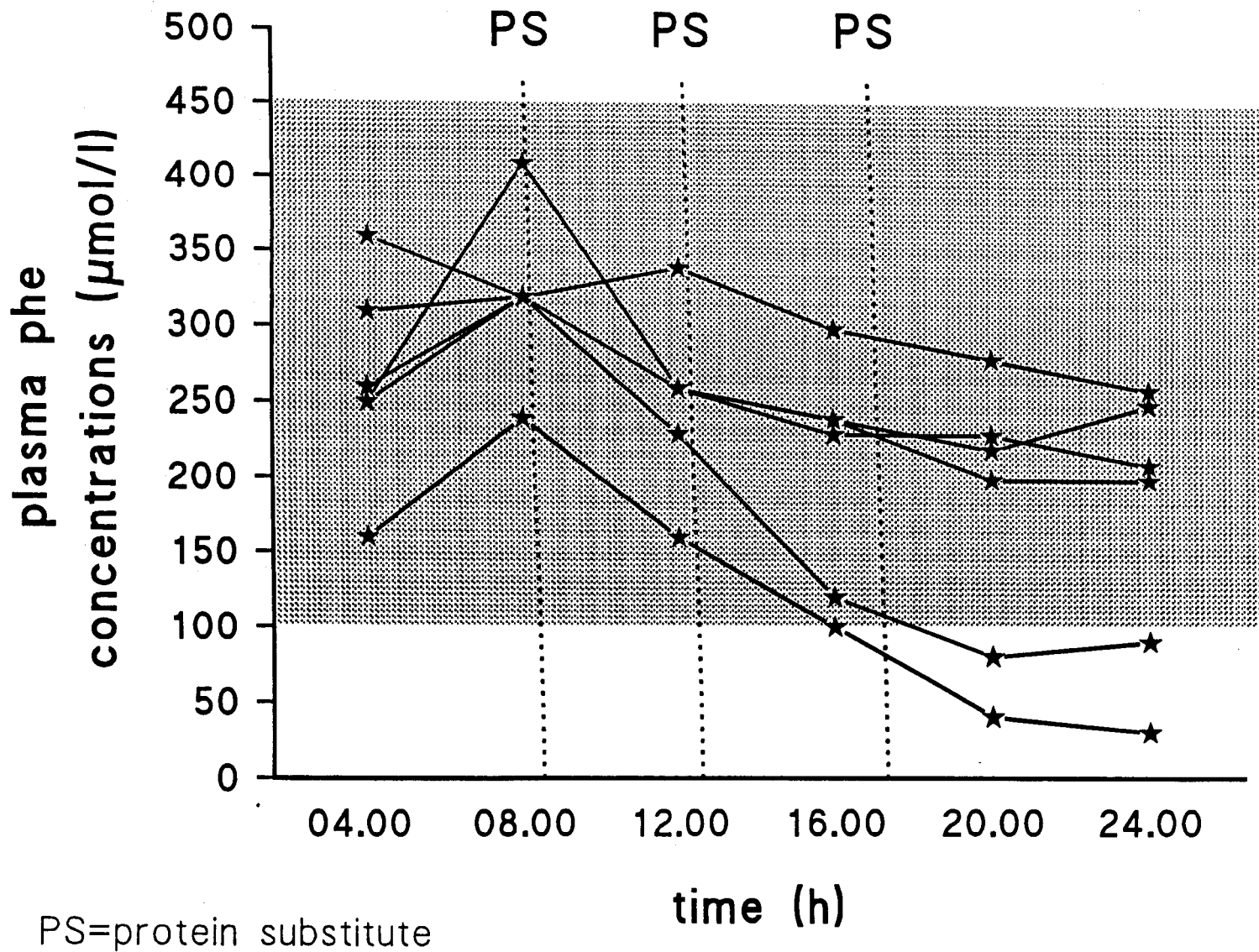
Fig 6.16 24h plasma phenylalanine profile for all subjects for Protocol B (when 33% protein substitute administered between 7.00-8.00h, 15.30-16.00h and 21.00h-22.00h)
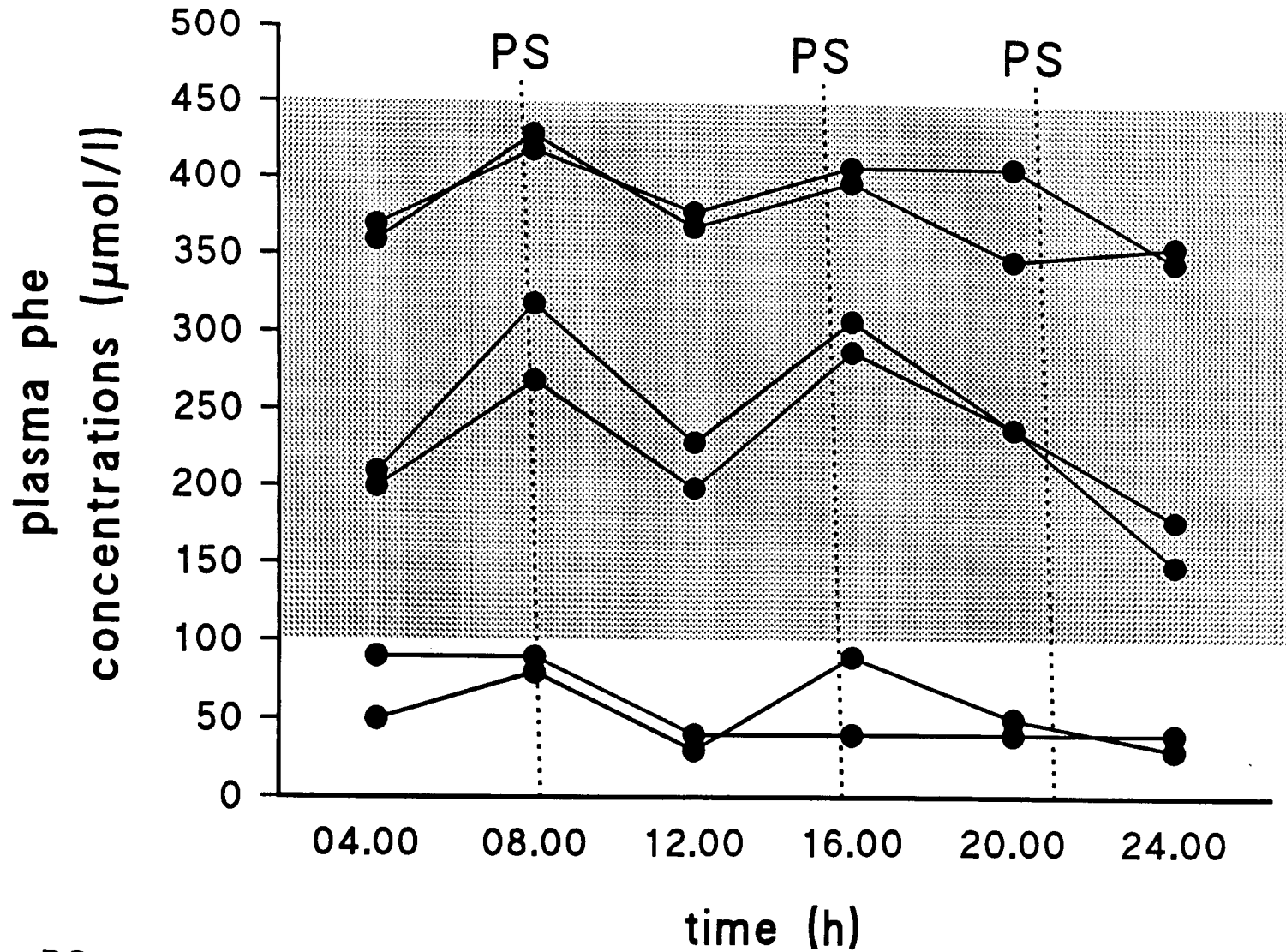
PS=protein substitute

Fig 6.17 24h plasma phenylalanine profile for all subjects for Protocol C (when 16.5% protein substitute administered at 4.00, 8.00h, 12.00, 16.00h, 20.00h and 24.00h)

PS=protein substitute
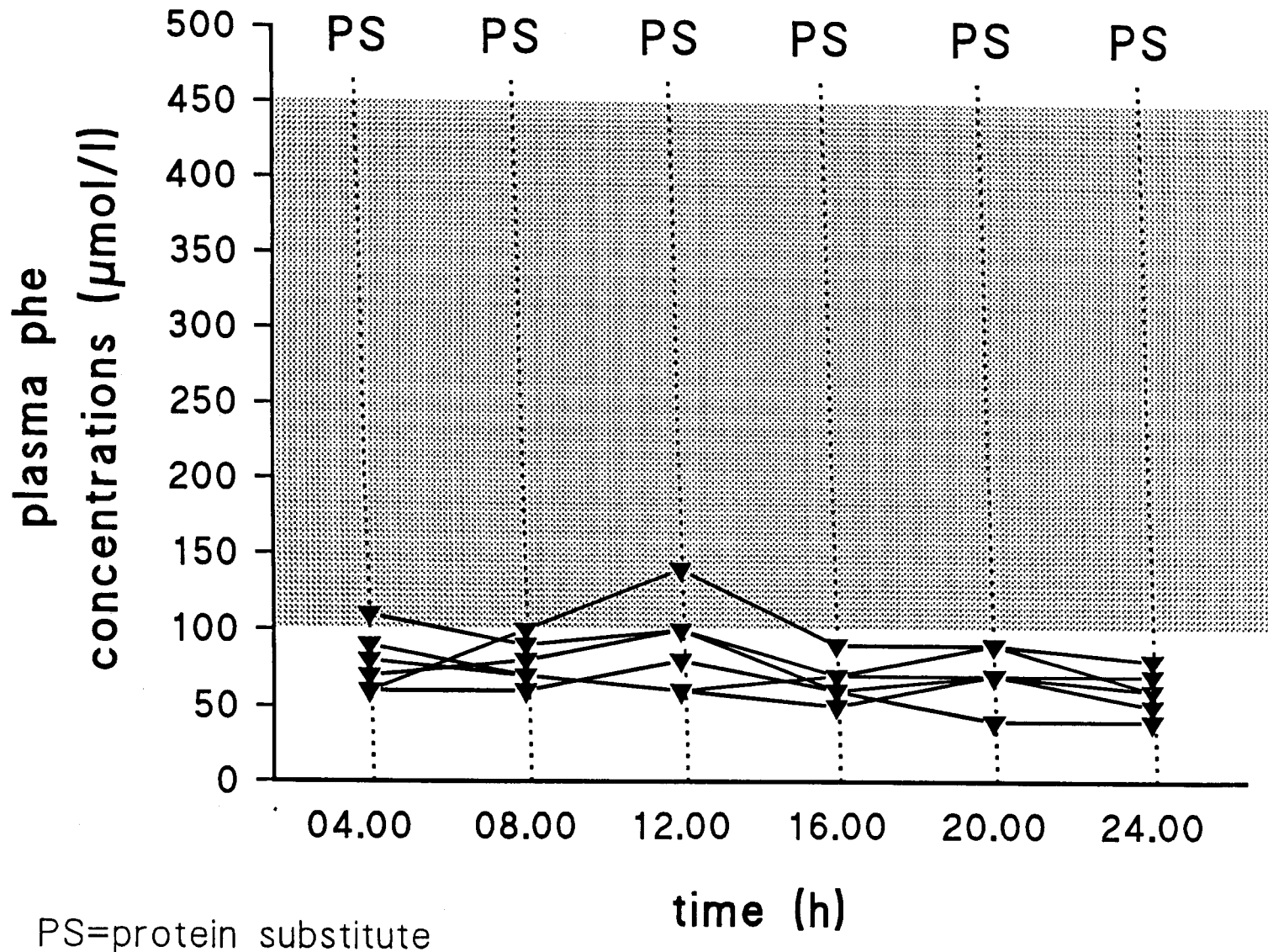
Fig 6.18 The change between highest and lowest plasma phenylalanine concentrations over 24 hours for all subjects in Protocol A, B and C
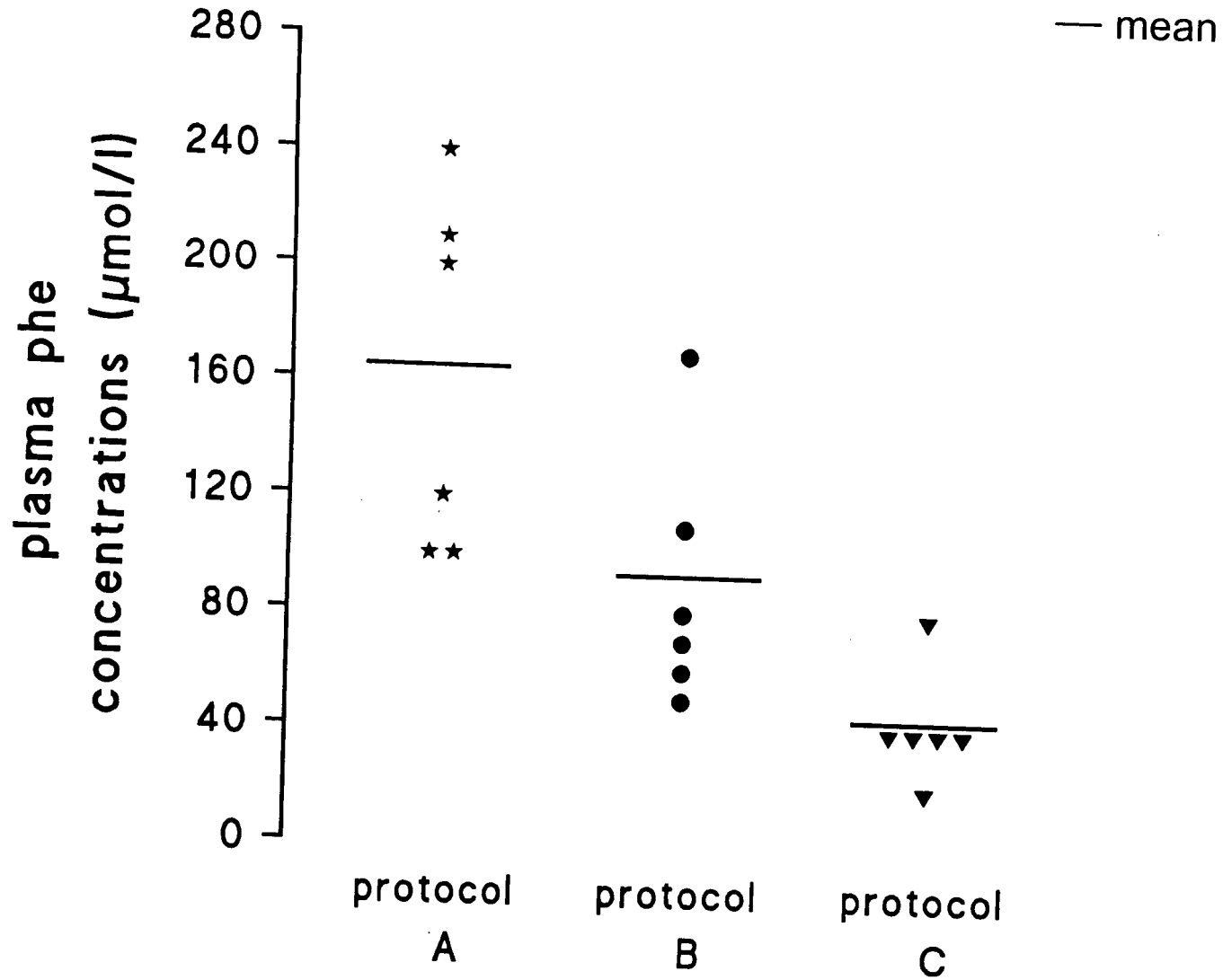
Chapter 7

Abnormal feeding behaviours
in phenylketonuria

7.1 Introduction

The low phenylalanine diet used in the treatment of PKU is one of the most restrictive and difficult of all diet therapies (Table 7.1). Given the central role that feeding and eating play in the life of a developing child, it is possible that such rigid dietary therapy may adversely affect feeding behaviour as well as nutritional status.

Table 7.1

Problems associated with a low phenylalanine diet

Factor	Problem
Variety:	**Limited**
Natural foods:	**Very restricted**
Protein substitute:	**Strong, bitter taste**
Low protein special foods:	**Dry, hard and bland**
Phenylalanine exchanges:	**Strict apportioning system**
Parent/carers:	**Have to ensure all protein substitute and phenylalanine exchanges are taken**

The effect of such limited diet therapy has not been studied in PKU. Therefore, the feeding behaviour of young children with PKU was investigated to identify feeding patterns and to determine the type and incidence of feeding problems.

7.2 Subjects and methods

7.2.1 Subjects

All children aged 1 to 5 years (mean 3.0 years) attending the PKU clinic were studied. There
were 12 girls and 3 boys. Each child was compared with an unrelated, age- and sex-matched
non-PKU control child. Controls were selected by each mother of the PKU children
identifying a friend of the same age and sex, living in similar housing and the same location.
None of the control children were known to have any medical problems and none were or
had been subject to any dietary restrictions.

All the PKU children were on a strict low phenylalanine diet as described in Chapter 2. The
median number of **50** mg phenylalanine exchanges allocated was **4** (range 3–7); total weight-
related protein intake from both protein substitute and phenylalanine exchanges was
3.0g/kg/day for children 1 year of age, and 2.5g/kg/day for children 2–5 years of age.
Thirteen children, according to their preference, took a protein substitute of L-amino acids
which incorporated carbohydrates, vitamins and minerals (i.e. *XP* Maxamaid; Scientific
Hospital Supplies); two preferred an L-amino acid supplement (i.e. Aminogran Food
Supplement; UCB Pharma), with supplementary vitamins and minerals.

The study was approved by the Committee on Medical Ethics of South Birmingham Health
Authority. Informed consent was obtained from all parents.

7.2.2 Methods

Assessment of feeding problems

Feeding assessment questionnaire

The mothers of all the children completed a feeding assessment questionnaire (Harris and Booth, 1992) administered by one researcher (Appendix 7.1). The questionnaire examined maternal perceptions of the incidence and type of feeding problems, considering appetite, gastrointestinal symptoms and feeding environment as well as feeding behaviour, feeding patterns, feeding skills, parental management and food preferences. A subjective five point positive-negative rating scale was used to determine parental feelings about children's mealtimes and appetite. A supplementary questionnaire was administered to mothers of PKU children to examine their perception of ease or difficulty of protein substitute administration (Appendix 7.2).

Video-recorded meals

A video recording of each child eating a midday meal at home was made by the same researcher (AM). This did not include the administration of the protein substitute. All communication between the carer(s) and child was recorded. The video camera was in open view, but carers were asked to continue their normal mealtime activity. The investigator visited each house one hour prior to recording so the children could become used to her presence. The video-recorded meals were analysed using a scored category system based on the observed behaviour of both mother and child during the mealtime. (Thomas and Harris, 1993) (Appendix 7.3). The analysis comprised the number of times the child became distracted from the meal, the manner in which the child's attention was redirected back to the meal, the number of mouthfuls eaten during the meal, child and parental vocalisations; and

parental intervention during the mealtime. Child behaviour during mealtime and meal duration to the nearest minute was also documented.

The video analyses were carried out by one observer. In order to verify reliability of the "observer" scoring technique, analysis of a five minute "snapshot" of 10 of the videos (five with PKU and five control) was carried out by a second investigator blinded to the PKU control status of each subject. There was a high degree of correlation for individual fifteen variables checked, with a minimum correlation coefficient **of** $r = 0.8232$ and a maximum of $r = 0.9756$.

Dietary intake

Mothers recorded 3-day food intakes using household measurements such as tablespoons and teaspoons to describe portion sizes from Sunday to Tuesday inclusive. Mothers recorded only food actually eaten, cooking techniques and food brand. They returned nutritional labels of manufactured foods eaten. Nutritional analysis of food intake was calculated as described in Chapter 2.

Anthropometry

Anthropometric data were collected at the time of the video recording. Weight to the nearest 10g, was measured using portable electronic scales. Length was recorded to the nearest 1mm by measuring supine length by an infantometer in children less than 2 years, and standing height in children over **2** years using a Harpenden Stadiometer. Length/height measurements were then converted into z scores, which denotes units of standard deviation from the median.

7.3 Statistical analyses

Statistical analysis was by unpaired t-tests, Mann-Whitney and Fisher's exact test to compare the differences between the PKU and control group. The Pearson Product Moment Correlation Coeficient was used to compare the difference between the observer and independent observer for video scoring.

7.4 Results

7.4.1 Feeding assessment questionnaire

Children with PKU were perceived by mothers to have more feeding problems (Table 7.2). Gastrointestinal symptoms (constipation, diarrhoea, and/or abdominal pain) were more common in the PKU group (p <0.05) (Fig 7.1). However, mothers reported no differences in frequency of individual negative feeding behaviour such as gagging, pushing food away, or closing the mouth when offered food. Of 70 incidents of negative feeding behaviour reported, 41 were reported in the PKU group and 29 in the control group, but these differences were not significant.

Sweet foods were less popular in the PKU children and were disliked by almost half of the children studied (Table 7.3). **A** 5-point subjective positive-negative rating (with a score' of 1 being the highest) determining parental feelings and impressions about mealtime indicated that mothers of the PKU group felt their children were more difficult to feed. The PKU group scored a median of 3.0 (range 1–5); the control group a median of 1.0 (range 1–3); p <0.001. Using the same positive-negative rating scale, mothers of PKU children reported

that their children did not eat enough: PKU group median 3.0 (range 1–5); control group

median 1.0 (range 1–4) (p <0.001). Despite these differences, mothers of PKU children did

not indicate they found mealtimes specifically more stressful, tearful or hectic than the

control group.

7.4.2 Video analysis

Meal duration

The children in the PKU group were slower to feed. The median (range) meal duration for

the PKU group was 24 minutes (5–73 minutes) and for the controls, 16.0 minutes (10-35

minutes) (p <0.05). Sixty seven percent of the PKU group took over 20 minutes to eat their

midday meal compared with 20% in the control group (p <0.05) (Fig 7.2). Sixty percent of

the PKU group ate less than two mouthfuls per minute, but all of the children in the control

group ate more than two mouthfuls per minute (p <0.001).

Child feeding behaviour (Table 7.4)

The children in the PKU group were less likely to start eating their meal without prompting

(p <0.005). Although the PKU group were more easily distracted from the task of eating,

turned their head away or closed their mouth when offered food and expressed more

negative and less positive vocalisations about food, these differences were not significant.

Parental management and strategies (Table 7.5)

In both groups, most children ate at a table or high chair at mealtimes (PKU group 65%,

control group 87%). However, the children in the PKU group were more likely to be given

their midday meal separate from the rest of the family (PKU group **67%,**

control group 20%; p <0.05) (Fig 7.3). For every 10 minutes of the mealtime, there was less

parental feeding directed verbalisation e.g. asking if the food was nice, or good in the PKU

group, than in the control group (p <0.01).   There were more parental prompts reminding the

children to eat but less parental praise in the PKU group (p <0.05) (Fig 7.4).

Parental strategies more commonly employed in the PKU group to persuade children to eat

included bribery, distractions and threats, but with the exception of bribes (p <0.05), the

differences were not significant (Fig 7.5). Thirty three percent of mothers in the PKU group

used more than three strategies to persuade their children to eat during the observed

mealtime in the PKU group, compared with only 7% in the control group (p = 0.1686).

7.4.3 Dietary assessments (Table 7.6)

Although the mean energy intake, when compared to the estimated average requirement,

(DH, 1991) was higher in the control group than the PKU group, the difference was not

statistically significant. In the PKU group, protein substitute contributed a mean

intake of 34% (range 18–55%) of the energy. Children with PKU had fewer daily snacks;

ate smaller portions and were generally offered fewer foods at mealtimes. A mean of 21

different foods were consumed over 3 days in the PKU group compared with 36 in the

control group (p <0.0001).

7.4.4 Protein substitute administration

Forty eight percent of the mothers of the PKU group said they had difficulty in persuading

their children to take the protein substitute and **only** 38% said their children completed all of

it every day. Sixty two percent of mothers supervised the protein substitute administration

and 19% said it took their children over one hour each day to complete, with one child taking as long as seven hours per day. Ninety one percent of the children took the protein substitute as a drink and 9% as a paste. Of the children taking it as a drink, as many as 48% still took it from an infant feeding bottle or beaker with teat attached as their parents could not persuade them to take it from an open cup or beaker. None of the children had their protein substitute administered via a gastrostomy or nasogastric tube.

7.4.5 Anthropometry

The PKU group of children had significantly lower weight z scores than the control group ($p < 0.05$), but there were no significant differences between the two groups for height z scores (Fig 7.6). Two children in the PKU group had a height z score below **-2.** There was a significant correlation between weight and energy intake in the control group ($p < 0.05$) (Fig 7.7), but this did not quite reach statistical significance in the PKU group ($p = 0.0895$) (Fig **7.8**).

7.5 Summary

This study is the first to define feeding problems in young children with PKU. Forty seven percent of the mothers perceived their children to have at least **3** feeding difficulties. Principal problems perceived and observed were slowness to feed; a poor appetite; a dislike of sweet foods; and a limited variety of foods consumed. Parents also perceived their children to have more gastro-intestinal symptoms. It was observed that carers of PKU children resorted to more mealtime coercive strategies to persuade their children to eat, used less verbal encouragement at mealtimes and were more likely to feed their children in

isolation. Particular difficulties were experienced with the administration of protein substitute. Although PKU children were lighter and smaller than control children, only two PKU children were stunted (height age less than **2** standard deviations of reference value). However, both of these children had at least one small parent, and neither had low energy intakes. This study has therefore identified a number of feeding problems in young PKU children, some of which may be precipitated by the administration of protein substitute.

Table 7.2

Difficulties as perceived by mothers

Problem	PKU (%) n = 15	Control (%) n = 15	p value
Poor appetite	8 (53)	1 (7)	< 0.05
Eats limited variety of foods	10 (67)	4 (27)	NS
Slow to feed	10 (67)	3 (20)	< 0.05
Cannot chew	2 (13)	0 (0)	NS

Table 7.3

Food dislikes

FOOD	PKU (%) n = 15	Control (%) n = 15	p value
Vegetables	3 (20)	1 (7)	NS
Bread *	5 (33)	1 (7)	NS
Fruit	2 (13)	0 (0)	NS
Sweets/chocolate *	7 (47)	0 (0)	< 01
Puddings *	9 (60)	0 (0)	< 0.001
Cakes/biscuits *	6 (40)	0 (0)	< 0.05

* low protein in the case of children with PKU

Table 7.4

Child feeding behaviour during midday meal: results *of* video analysis

	PKU n = 1 5	Control n = 1 5	p value
Number of children needing parental prompting at beginning of meal to start eating	10	1	< 0.005
Median (range) number of times child turns head away when proffered food per 10 minutes	0 *(0-5)*	0 (0-3.3)	NS
Median (range) number of times child closes mouth when proffered food per 10 minutes	0 (0-2.7)	0 (0-1.3)	NS
Median (range) number of times child is distracted from task of eating per 10 minutes	2.9 (0-6.7)	2.1(0-6)	NS
Mean (range) number of positive vocalisations from child per 10minutes	0.6 (0-3.6)	0.8(0-10)	NS
Median (range) number of negative vocalisations from child per 10minutes	1.0(0-6)	0.7(0-5)	NS

Table 7.5

Parental meal time strategies and vocalisations: results of video analysis

	PKU n = 15	Control n = 15	p value
Parental vocalisations			
Median (range) parental feeding directed vocalisations per 10 minutes	3.7 (0.5-22)	12.0 (2.3-29)	<0.05
Median (range) parental feeding direct prompting per 10 minutes	6.3 (0.5-18.7)	2.7 (0.8-13.5)	NS
Median (range) parental feeding directed praising per 10 minutes	0.4 (0-4)	1.3 (0-5.9)	<0.5
Parental strategies			
Median (range) number of mealtime strategies per meal	2.0 (1-5)	1.0 (1-3)	NS
Median (range) number of incidents of parental bribing per meal	0 (0-4)	0 (0-3)	NS
Mean (range) number of parental distractions per meal	0 (0-6)	0 (0-2)	NS
Median (range) number of parental threats per meal	0 (0-9)	0 (0-0)	NS

Table 7.6

Food intake

	PKU n = 15	Control n = 15	p value
Median % EAR for Energy * (range)	99% (719 – 179)	122% (93 – 154)	NS
Mean number of snacks (SD)	1 (0.89)	2.6 (0.5)	<0.0001
Median number of foods at mealtimes (range)	2 (1 – 4)	4 (2 – 7)	<0.01

* DH, 1991

Fig 7.1   **Gastrointestinal symptoms in PKU and control group reported by mothers**

■ = PKU
□ = control
p<0.05

subjects (%)

100
80
60
40
20
0

vomiting   constipation   diarrhoea   pain
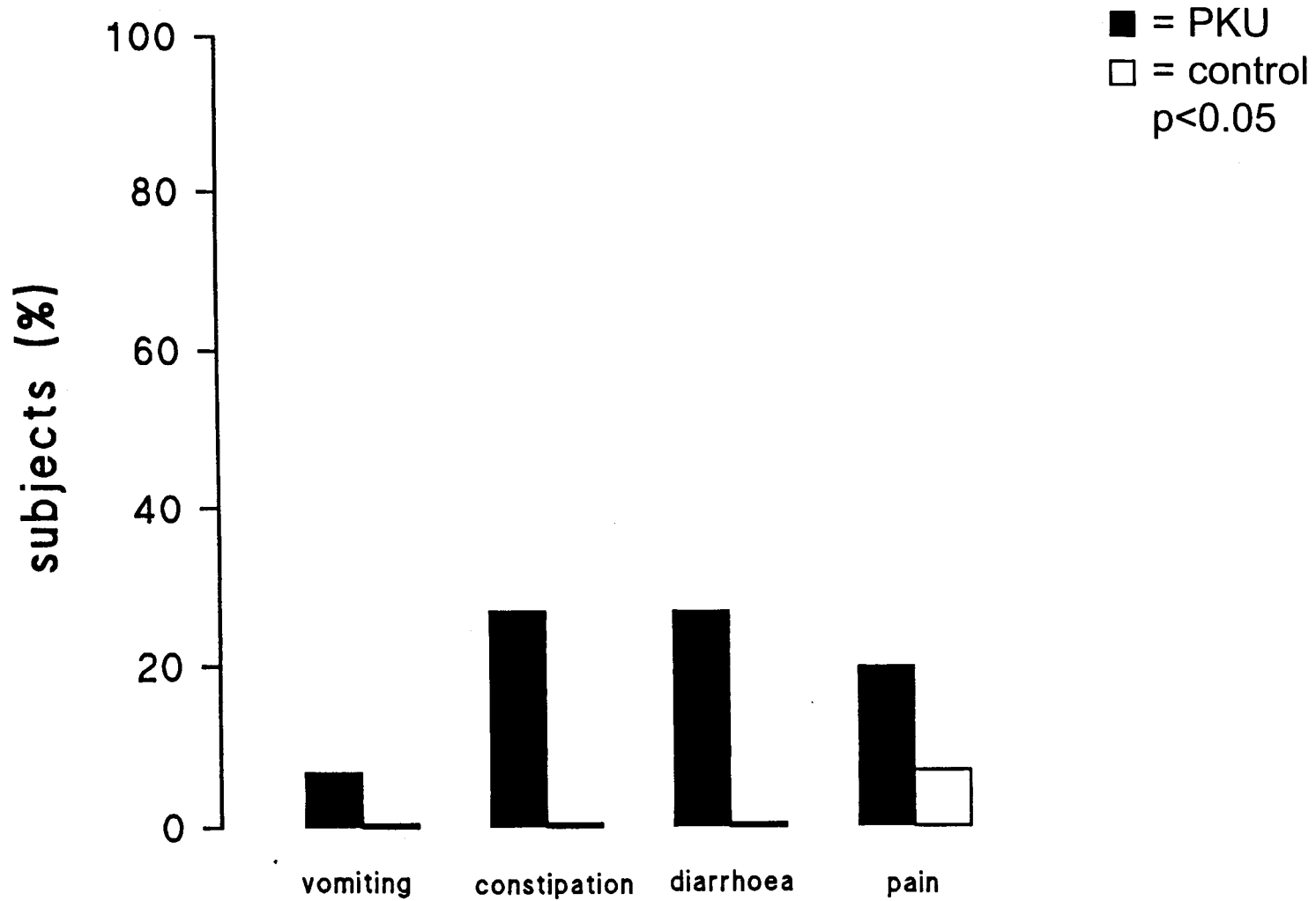
Fig 7.2   Duration of midday meal of PKU and control group
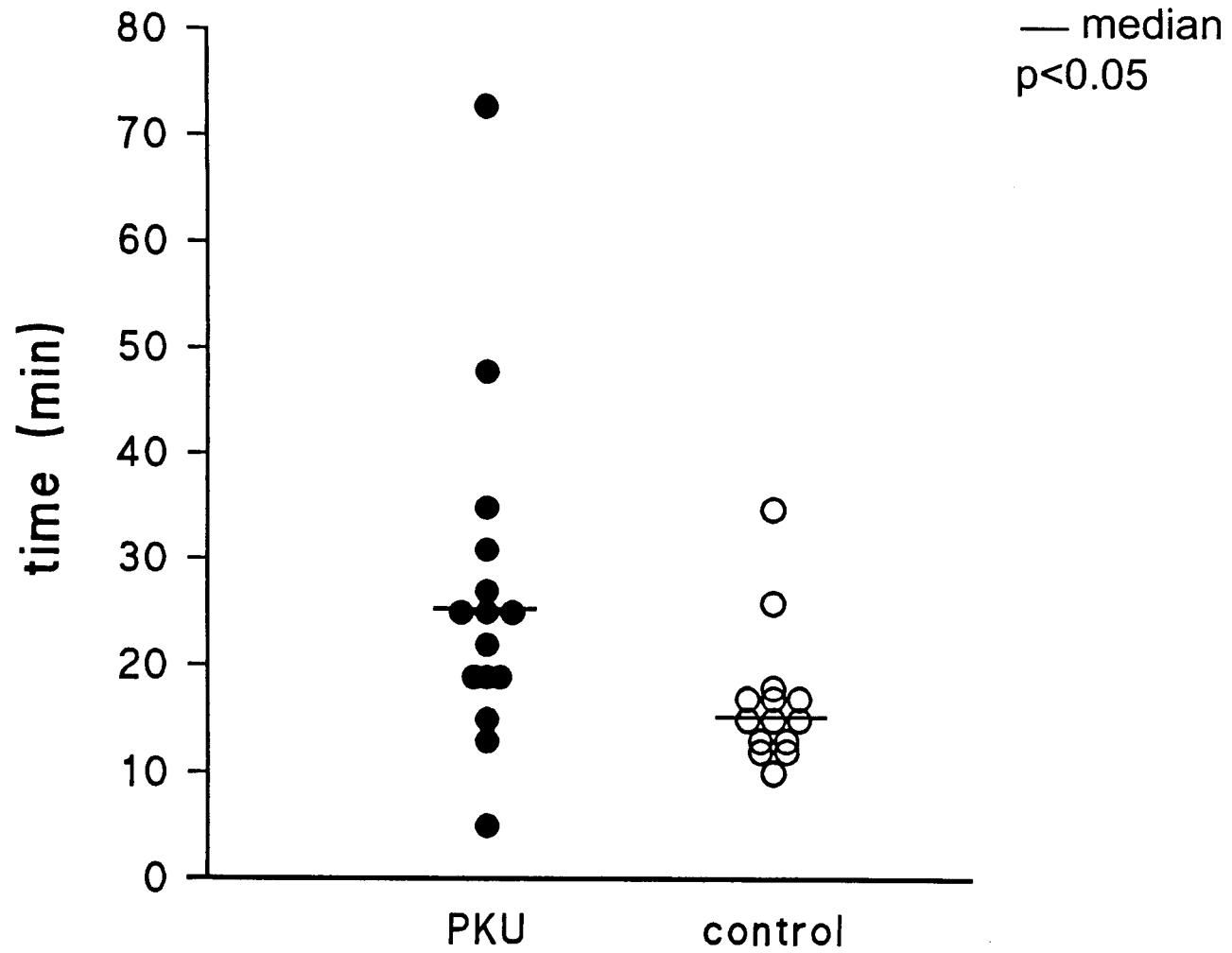
Fig 7.3 **Mealtime setting for PKU and control group**
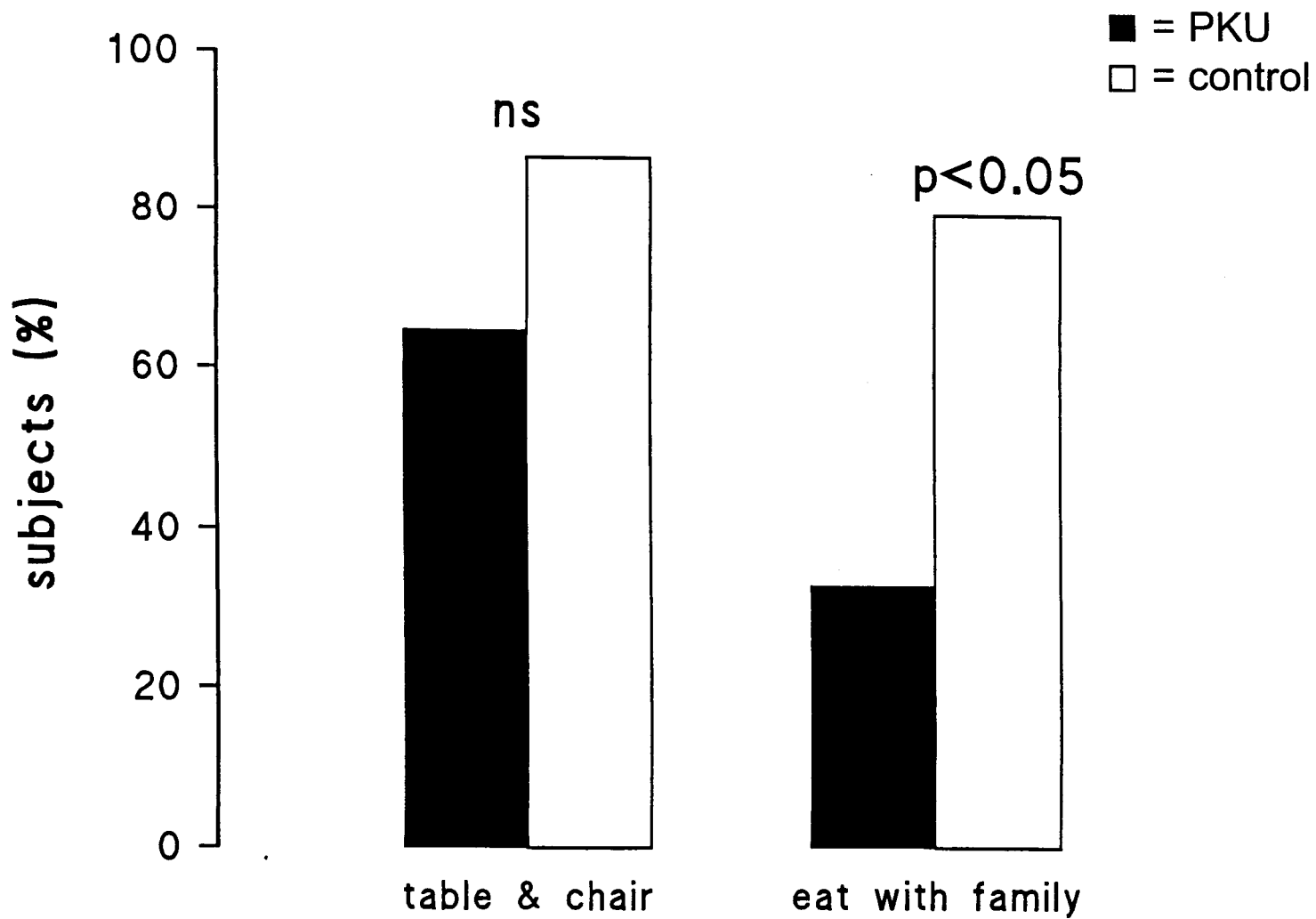
Fig 7.4 Recorded mealtime parental conversation for PKU and control group
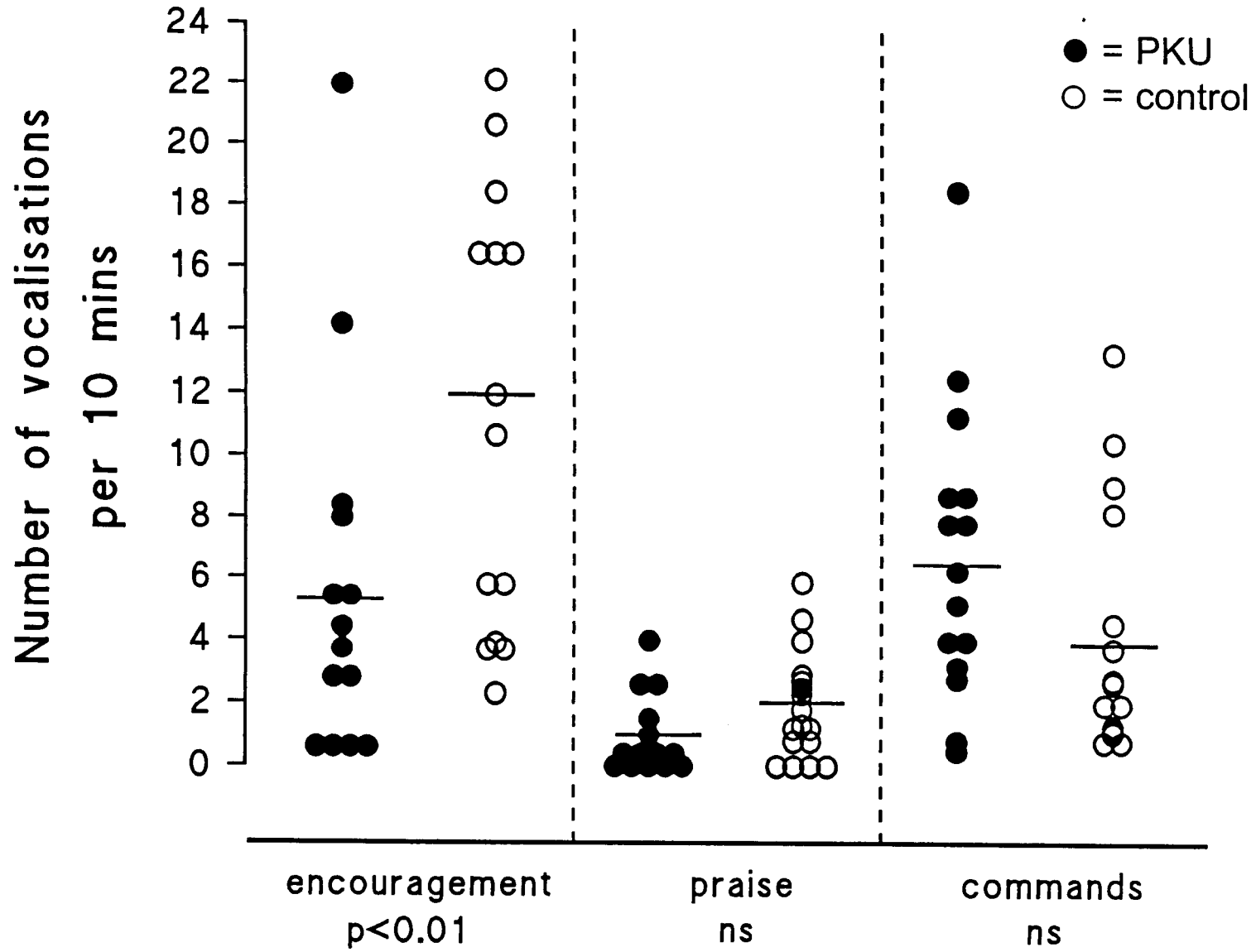
Fig 7.5

Parental mealtime strategies for PKU and control group

■ = PKU
□ = control

p<0.05

number per meal

any strategy   bribes   distractions   threats
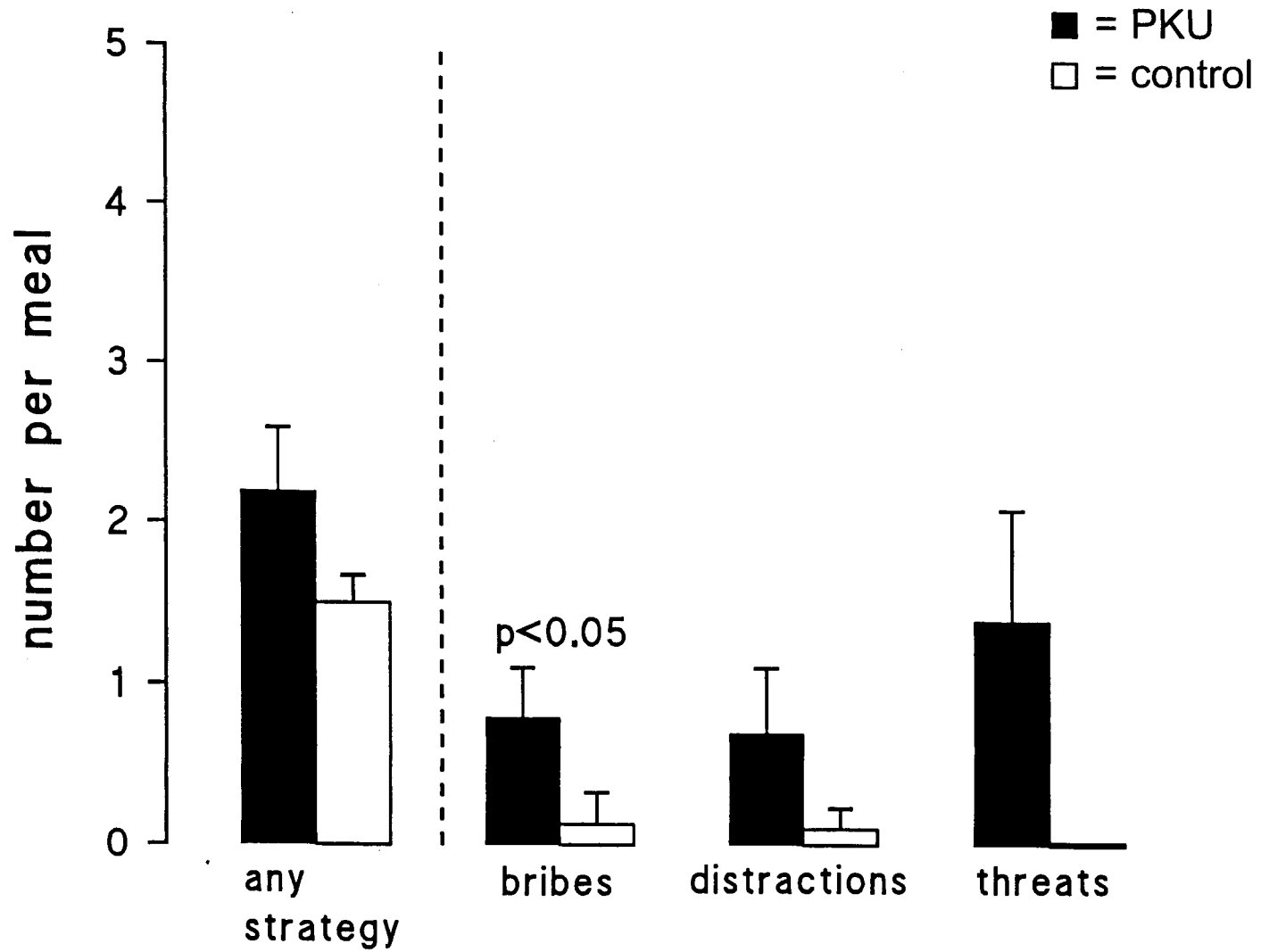
Fig 7.6   **Weight and height z scores for age for PKU and control group**
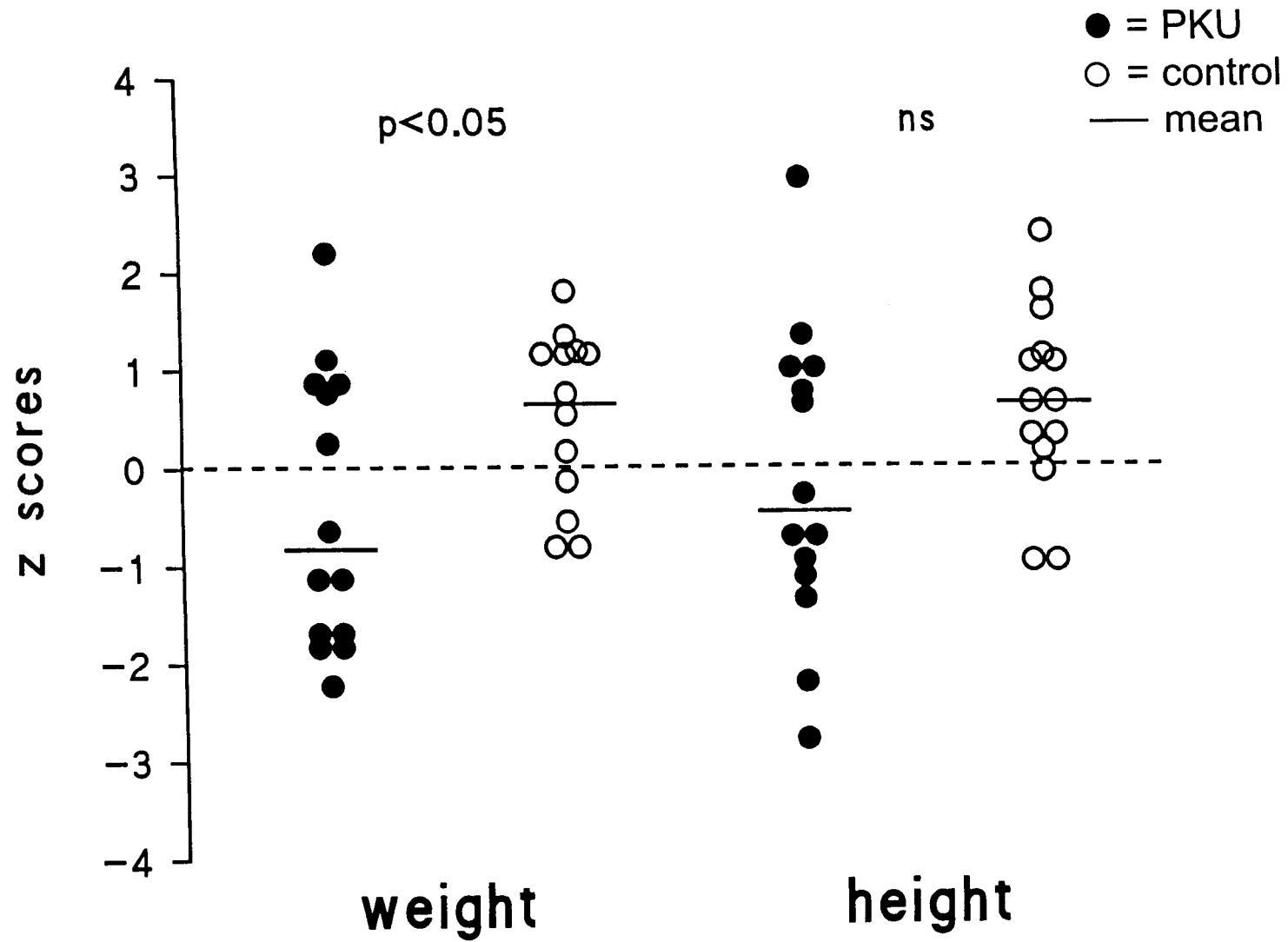
Fig 7.7 **Correlation between weight z scores and % EAR for energy for control group**

r= 0.6003
p<0.05

weight z score

energy intake (% of EAR)
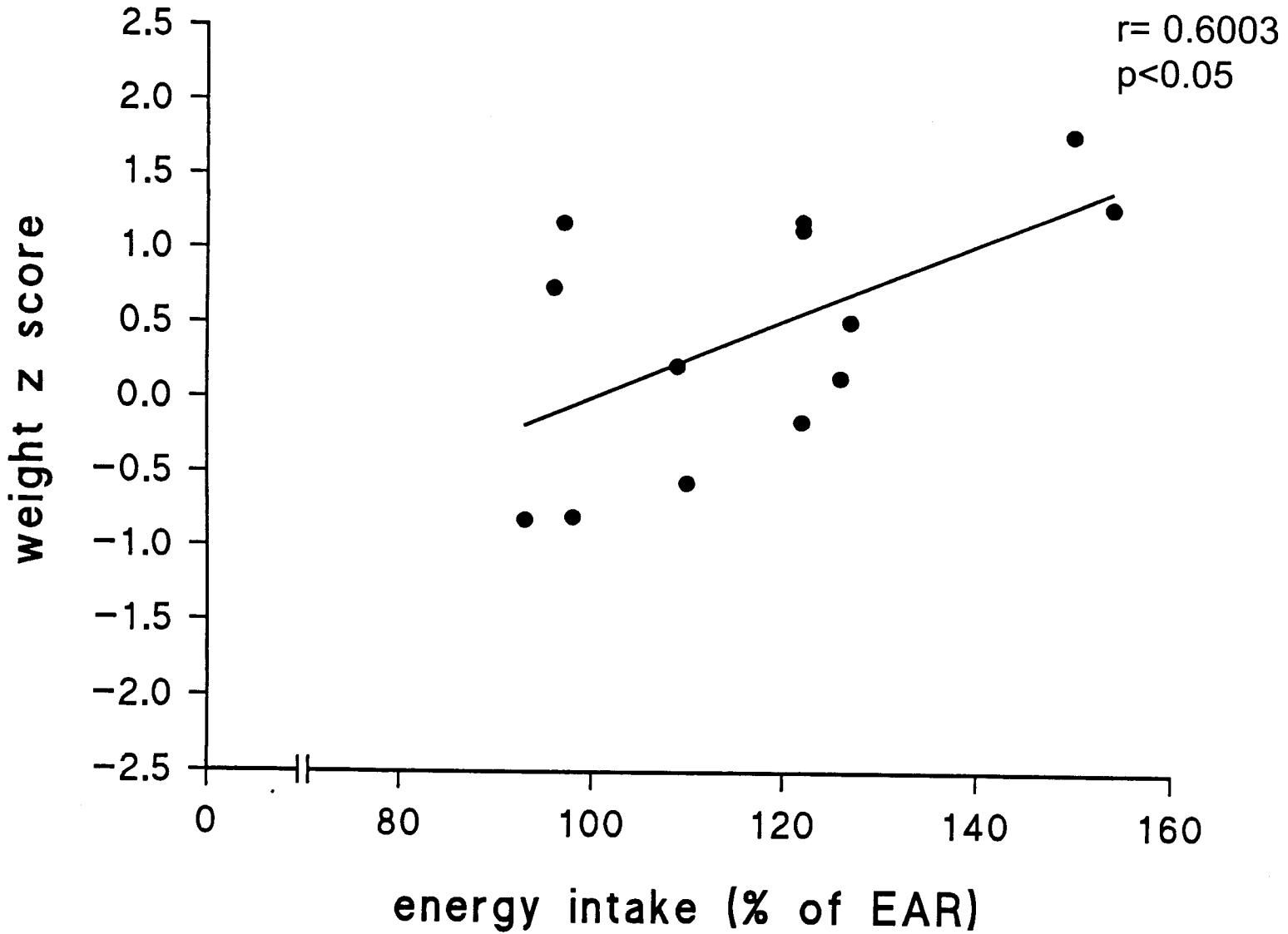
Fig 7.8   Correlation between weight z scores and % EAR for energy for PKU group
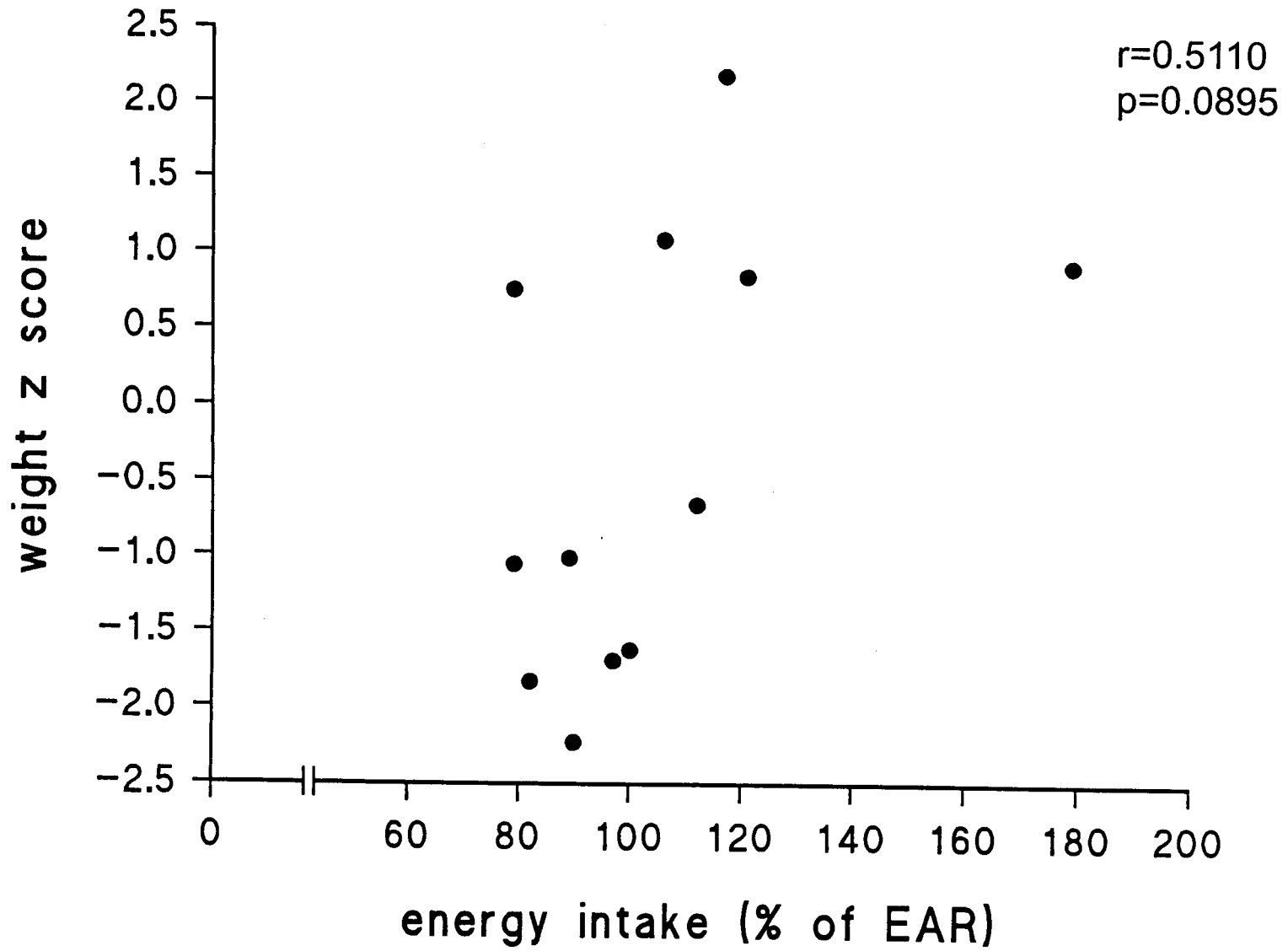
Chapter 8

Discussion

8.1 Principal findings

This work reports a series of original studies principally investigating the effects of diet on a) variability in blood phenylalanine concentrations and b) feeding behaviour in young children with PKU. Many of the studies have produced unexpected results, but provide a greater understanding of the factors affecting blood phenylalanine and the dietary problems faced by parents and young children. The main findings are summarised as follows:

- There was wide 24-hour variability in plasma phenylalanine concentrations in PKU that was not reflected in single early morning phenylalanine observations.

- Twenty-four hour variability was more influenced by the timing and dosage of protein substitute than total energy **or** excess natural protein intake from 'freely' allowed foods.

- Manipulation of timing and dosage of protein substitute during daytime hours did not improve 24-hour plasma phenylalanine profile. However, repeated administration of protein substitute every four hours day and night markedly reduced phenylalanine variability and produced stable and lower phenylalanine concentrations.

- Fruits and vegetables containing phenylalanine between **51-100** mg/100g did not adversely affect control and so can be incorporated 'freely' into the diet.

- New phenylalanine analysis for potatoes allowed a substantial increase in portion size, and prolonged testing indicated this did not adversely affect phenylalanine concentrations.

- **A** number of feeding problems was identified in young PKU children, some of which may be precipitated by administration of protein substitute.

The discussion is divided into three parts:

- Interpretation and implications of results.

- Strengths and weaknesses of individual studies.

- Unanswered questions and future research.

8.2 Interpretation and implication of research results

8.2.1 Variability in blood phenylalanine concentrations

In this thesis, the two studies **(Chapter 2 and 5)** examining plasma phenylalanine variability are the first to provide detailed knowledge about the wide 24-hour variability of plasma phenylalanine concentrations in such large groups of subjects in PKU. They show that phenylalanine concentrations do not stay within narrow target ranges over 24 hours for many patients. They also demonstrate that single early morning specimens in any one 24-hour period give an incomplete and unrepresentative picture of phenylalanine control for many children. They add further knowledge to the early work conducted by Güttler who measured twice daily phenylalanine concentrations in two children (Güttler *et al,* 1969); but conflict with those of van Spronsen who found little fluctuation in plasma phenylalanine concentration over five hours in eight subjects (van Spronsen *et al,* 1993).

In **chapter 2,** the study demonstrated large diurnal fluctuations of plasma phenylalanine concentrations in a group of well-controlled PKU children. It showed that blood samples before breakfast did not always reflect the highest plasma phenylalanine concentrations of the day. It also showed that almost half of all evening plasma phenylalanine concentrations were less than $100\mu mol/l$ in children who had taken 65% or more of their protein substitute

by the time of their evening meal, despite having morning blood phenylalanine concentrations within the MRC recommended range (MRC, 1993b). For the study group overall, there was significantly greater variability in evening plasma phenylalanine concentrations than for morning concentrations.

This study was limited in that phenylalanine concentrations were measured at only two time points within each 24-hour period. It might therefore have failed to detect considerable and therapeutically important inter-individual variation in phenylalanine concentrations throughout a 24-hour period. These findings and shortcomings prompted the need for the 24-hour blood phenylalanine study **(Chapter 5).** This study demonstrated wide fluctuations in plasma phenylalanine concentrations throughout a 24-hour period. The median difference between highest and lowest concentrations was 155 μmol/l. Peak concentrations occurred before midday in only 69% of cases, but the lowest almost always occurred in the afternoon or evening. There was no correlation with age, and median variation in phenylalanine concentrations was similar in young and older patients. These findings have recently been confirmed by Francois and Diels who demonstrated wide variability in blood phenylalanine concentrations in a group of 10 patients with PKU aged from 12–30 years (Francois and Diels, 1998).

Hypophenylalaninaemia

The 24-hour variability study identified the range of time during which phenylalanine concentrations lay outside the MRC guidelines in 'well controlled' children. It demonstrated that if an early morning phenylalanine concentration was just above the MRC lower limit, and therefore considered satisfactory, it could be less than 100 μmol/l for a significant time

during the 24 hours. It was of particular concern that the study identified a small number of children who had phenylalanine concentrations less than 30 μmol/l for up to seven hours within a 24-hour period.

Although there is no evidence that hypophenylalaninaemia is detrimental to long term neurological outcome, or that transient hypophenylalaninaemia is detrimental to intelligence quotient, it is only within the last four years in the UK that phenylalanine concentrations have been maintained within such narrow and low limits. There is some concern that long term low plasma phenylalanine concentrations may affect IQ and growth. In data from the National Phenylketonuria Register, Smith and co-workers showed that IQ fell by four points for each five months during which measured phenylalanine concentrations were below 120 μmol/l (Smith *et al,* 1988). However, the analyses of outcome of these results may relate largely to morning rather than evening concentrations, although blood-sampling timing was not specifically reported. In addition, plasma phenylalanine was analysed primarily from bacterial inhibition assays or paper chromatography, which are not particularly accurate at low concentrations. Some studies from the early 1970's reported poor growth linked to overzealous restriction of dietary phenylalanine and low plasma phenylalanine concentrations in the first few months of life (Hanley *et al,* 1970; Smith and Waisbren, 1971). Furthermore, there have been three studies from Europe reporting improved growth in infants and young children with PKU (Dhont *et al,* 1995; Schaefer *et al,* 1994; Verkerk *et al,* 1994). In the Netherlands, it has been shown that height z scores in children with PKU at the age of 3 years were less than reference values. For boys, this reflected an average decrease of 30 mm in expected height (Verkerk *et al,* 1994). These authors' hypothesised treatment regimens aiming at maintaining lower phenylalanine

concentrations may lead to adverse effects on growth. Even so, they found no direct correlation between the percentage of phenylalanine concentrations less than 120 µmol/l and poor growth, although they did not appear to correlate the actual extent by which phenylalanine concentrations fell below 120 µmol/l with growth. They also recognised that other periods of hypophenylalaninaemia may have remained unidentified.

Hyperphenylalaninaemia

Phenylalanine concentration only rose above MRC working group upper reference ranges in subjects who had relatively high starting point concentrations (MRC, 1993b). In the 24-hour phenylalanine variability study, with the exception of one subject, higher than MRC recommended concentrations only occurred in teenagers who aimed to maintain their phenylalanine concentrations between 100 and 700 µmol/l. Phenylalanine concentrations over 700 µmol/l for a significant proportion of a 24-hour period may have consequences for neuropsychological performance. Short-term concentrations over 800 µmol/l have been associated with prolonged performance times on neuropsychological tests of higher integrative function (Krause *et al,* 1985). **A** phenylalanine concentration over 600 µmol/l on the day of neuropsychological testing has also been associated with a sustained attention deficit and difficulty with calculation tasks (de Sonnerville *et al,* 1990).

The 24-hour plasma phenylalanine variability study clearly demonstrates that single-point, early morning, phenylalanine concentrations were misleading and provide an incomplete picture of phenylalanine control in PKU. If it is not possible to lessen 24-hour plasma phenylalanine variability by dietary manipulation; it may be necessary to consider annual

24-hour plasma phenylalanine profiles in children with PKU to estimate the extent of individual phenylalanine variability and help with interpretation of phenylalanine concentrations.

8.2.2 Dietary factors affecting variability in blood phenylalanine concentrations

Timing and dosage of protein substitute intake

In this thesis, it has been clearly demonstrated that plasma phenylalanine variability was strongly influenced by the timing and dosage of protein substitute intake. Administration of 25% or more of daily protein substitute caused a decline in phenylalanine concentrations. If further protein substitute was not consumed for several hours, there was an increase in plasma phenylalanine. In the study on factors affecting variability in plasma phenylalanine **(chapter 2),** subjects who had taken *65%* or more of their protein by the time of their evening meal showed a fall in plasma phenylalanine concentrations during the day. In contrast, subjects who delayed most or all of their protein substitute until after their evening meal showed an increase in plasma phenylalanine concentration through the day.

In the 24-hour variability plasma phenylalanine study **(chapter 5),** eighty two percent (n = 13) of all the children took the protein substitute three or more times daily, but only 44% (n = **7**) took it at the recommended three to four hour intervals with meals. In the latter group there appeared to be persistent depression of phenylalanine concentrations during the daytime and early evening, but increased concentrations overnight. Younger children in particular were more likely to take the protein substitute in this way. The other **38%** of the subjects (n = *6*), mainly older patients, took a late evening dose of protein substitute. They

had relatively small changes in phenylalanine concentration overnight (-60 µmol/l to +30 µmol/l).

When timing and dosage of protein substitute was altered during the day, the plasma phenylalanine pattern changed, but there was no reduction in plasma phenylalanine variability. In contrast, administration of protein substitute in equally divided doses throughout 24-hours reduced plasma phenylalanine variability to a rate almost equivalent to that of the normal physiological position. In addition, administration of protein substitute every 4 hours appeared to suppress phenylalanine concentration to the extent that almost all were below 100µmol/l. Although only three patients were studied, no phenylalanine concentration fell below the normal lower limit, which was different from the situation when protein substitute was administered in only **3** or 4 doses during daytime hours only. Continual administration of protein substitute day and night may therefore improve phenylalanine tolerance. However, this has not been specifically tested. If similar results were obtained in a larger group of patients, and phenylalanine concentrations were not to fall below lower limits of normal, it may be possible to reduce the lower range of blood phenylalanine concentrations permitted in PKU.

Explanatory mechanisms for action of protein substitute on plasma phenylalanine concentrations

It is likely that administration of protein substitute helps promote protein synthesis. The proteins of the body are continually being broken down and replaced. Tissue protein breakdown occurs at a more or less constant rate throughout the day. *An* adult catabolises and replaces about 3-6g of protein/kg/body weight per day (Bender, 1997). In the fed state when feeding normally, there is an abundant supply of metabolic fuel and the rate of protein

synthesis increases, exceeding that of breakdown. This causes a net increase in tissue protein, and replaces what is lost when in a fasting state. In the fasting state, this leads to a net loss of tissue protein by liberating amino acids, including phenylalanine, into the plasma pool, to supply a source of energy. In healthy individuals, these amino acids are largely oxidised. In people with PKU, however, phenylalanine cannot be hydroxylated, and so the plasma phenylalanine concentration increases. It is interesting to note that phenylalanine concentrations still increase during the day, even when no protein substitute is administered but a supply of dietary energy is maintained. Millward and Rivers give a model for the utilisation and influence of dietary amino acids (Millward and Rivers, **1988).**

It is possible that administration of protein substitute in a single daily dose, particularly when given in the form of L-amino acids, led to the extremes in variability of plasma phenylalanine concentrations seen in **Chapter 5.** L-amino acids are absorbed rapidly. In a study on ten normal men, administration of a single dose of L-amino acid resulted in an initial rise in plasma amino acids. However, after **90** minutes, there was a rapid decline in phenylalanine and, by 240 minutes, the concentrations had fallen below the baseline figure (Gropper, Gropper and Acosta, **1993;** Gropper and Acosta, **1991).** In addition, there is evidence that amino acids given in single doses lead to increased oxidative utilisation (Mönch *et al,* **1996),** increases in nitrogen excretion, symptoms such as vomiting, dizziness, nausea, fullness, diarrhoea and headaches, and in non-PKU adults, post-prandial hypoglycaemia.

Explanatory mechanism for effect of 4 hourly administration of protein substitute intake on plasma phenylalanine concentrations

It is probable that continual feeding stimulates protein synthesis day and night, thereby increasing efficiency of protein utilisation (Millward and Rivers, 1988). It has been shown in a group of malnourished children achieving catch-up growth during nutritional rehabilitation, feeding every 4 hours, that efficiency of protein utilisation was very high (Ashworth and Millward, 1986; Chan and Waterlow, 1966). Similarly, in rats fed every 4 hours, growth is more rapid and protein utilisation more efficient than in *ad lib* fed rats, which exhibit a diurnal pattern of intake (Obled, Arnal and Fauconneau, 1975).

The only other potential operative factor, when protein substitute was given day and night, was that the carbohydrate added to protein substitutes increased the energy intake overnight. Each dose of protein substitute provided 10 to 25g carbohydrate when protein substitute was administered during the day only; and 9 to 13g when protein substitute was given throughout the day and night. Although it is unlikely the carbohydrate played a major role in variability in blood phenylalanine concentrations, it probably enhanced utilisation of amino acids in protein synthesis.

Therefore, protein substitute is able to suppress plasma phenylalanine concentrations, probably by enhancing protein synthesis. Continual administration every 4 hours day and night is able to produce a lower and stable 24-hour plasma phenylalanine profile. This may possibly help to improve phenylalanine tolerance indirectly.

Dietary phenylalanine intake

The studies forming the basis of this thesis have demonstrated that despite dietary anomalies within the PKU dietary system, which masked a significant increase in the intake of dietary intake of phenylalanine, there was no proof to support the suggestion that better phenylalanine control could be achieved by making the dietary system more rigorous. Therefore, there appears to be no need to incorporate some of the 'free' foods into the phenylalanine exchange system.

It is perhaps surprising that excess or extra phenylalanine from 'free' fruit and vegetables did not affect phenylalanine concentrations. In addition to daily allocated natural protein in the form of 50 mg phenylalanine exchange, free foods increased natural protein intake by 49% and phenylalanine by **31%.** A wide day to day variation in natural protein intake was seen, but this effect was substantially reduced when phenylalanine intake was calculated. Free vegetables were responsible for the majority of excess natural protein intake, and these included cauliflower, tinned tomatoes, fried mushrooms and onions.

Possible mechanisms for lack of effect of extra phenylalanine from 'free' fruit and vegetables on plasma phenylalanine concentrations

There are four possible explanations why excess phenylalanine from 'free' fruit and vegetables did not affect blood phenylalanine concentrations.

1) Phenylalanine from fruits and vegetables may not be efficiently absorbed. It is known that vegetable protein is the least digestible of all proteins and a correction factor of only *85%* is given for digestibility for diets based mainly on vegetables and unrefined carbohydrate (Thomas, 1994). It may also be possible that the non-starch polysaccharide

content of some of the fruits and vegetables may reduce apparent digestibility by up to

10% and increase nitrogen excretion in the faeces (Thomas, **1994).**

2) Diets may be lower in phenylalanine than calculated. This may be why there is no

apparent effect of excess intake on blood phenylalanine concentrations. Due to a lack of

contemporary and comprehensive phenylalanine data of vegetable composite dishes, it is

impossible to accurately calculate the phenylalanine content of the **PKU** diet, so it is

necessary to use some protein analysis to estimate phenylalanine intake. Children with

PKU eat many convenience composite vegetable items, such **as** vegetable nuggets and

fingers, for which there is no phenylalanine analysis, and vegetables provide less

phenylalanine (per **g** of protein) than other basic foods. Phenylalanine intake may

therefore be overestimated.

3) The children ate more phenylalanine from free foods, which would increase their energy

intake. This may have enhanced protein utilisation.

4) Even with a generous 'free' food system in **PKU,** the mean daily phenylalanine intake of

the subjects in the factors affecting the variation in plasma phenylalanine study **(chapter**

2) is similar to, and indeed no greater than, two German studies documenting

phenylalanine intake (Wendel *et al,* **1990;** Schulz and Brumner, **1995).** It is lower than

that reported in an American study (Acosta *et al,* **1983)** (Table **8.1),** but in this study the

blood phenylalanine concentrations were much higher than considered acceptable in the

UK. Even in the German study on **1–6** year old children, the means of median

phenylalanine concentrations from 2 years of age were greater than the designated upper

limit of 360 µmol/l

Table 8.1 Intake of dietary phenylalanine

Phenylalanine intake from factors affecting variability in plasma phenylalanine study (chapter 2)			Acosta *et al*, 1997 Mean mg/kg	Acosta et al, 1983 Mean mg/kg	Acosta *et al*, 1983 Mean mg/day	Shulz *et al*, 1995 Median mg/day	Wendel *et al*,1990 Mean + (SD) mg/kg	Wendel *et al*, 1990 Mean mg/day
Age	Mean intake/ mg/kg	Mean daily intake mg/day						
1	28	271	27	30	285		28 (±7)	262
2	18	195			306		23 (±5)	276
3	18	255			351		20 (±5)	288
4	17	294			366		18 (±5)	288
5	-	-			404		17 (±5)	305
6	11	238		23	453		15 (±5)	316
7	13	316						
8	-	-						
9	19	524						
10	-	-						
11	-	-						
12	12	460						
13	-	-						
14	12	703				[		
15	-	-				[521 -		
16	14	870				[537		
17						(691 -		
18						(936		

Effect of phenylalanine intake on 24-hour plasma phenylalanine variability

Dietary phenylalanine (from exchanges and free foods) did not cause any significant increase
or peaks in phenylalanine concentrations. In the 24 hour blood phenylalanine variability
study, over 30% of subjects ate more than 50% of their natural protein in the evening and
this had no impact on blood phenylalanine control within 1 to 2 hours of meal completion.
Comparable results have been reported by others (van Spronsen *et al,* 1996). They showed
that plasma phenylalanine concentrations only increased above baseline by 10% to **18%**
when 50% of the total daily phenylalanine allowance was given and **8%** to 26%

with 75% of the total daily phenylalanine allowance. This is in contrast to non-PKU

individuals and may be because patients with PKU have a lower ratio of phenylalanine

intake relative to plasma pool size (van Spronsen *et al,* 1993). In 12 non-PKU and 12 adults

heterozygous for PKU, ingestion of a hamburger and milk shake meal, providing 1.0 g

protein/kg body weight significantly increased plasma amino acid concentrations over

baseline, reaching the highest mean value 3–5 hour after a meal occurred (Stegink, 1991).

Ashley and co-workers found that phenylalanine concentrations rose within one hour

following a high protein meal, but decreased after a carbohydrate enriched soup (Ashley *et*

al, 1982).

Dietary energy

Data from the factors affecting variability in plasma phenylalanine study **(chapter 2)**,

showed that energy intake fluctuated significantly within and between patients but did not

appear to influence plasma phenylalanine variability. This is surprising, as energy intake,

particularly from carbohydrate sources has been shown to be important in protein synthesis

(Motil *et al,* 1981).

The mean dietary energy intake met the EAR (DH, 1991), with carbohydrate providing the

majority of energy and fat providing a relatively low mean intake of **23%** of the energy.

This has been reported previously in PKU, and in fact, many patients have low cholesterol

levels (Schulpis *et al,* 1989; Galluzzo *et al,* 1985; Galli *et al,* 1991; Acosta *et al,* 1973) and a

poor long-chain fatty acid (LC-PUFA) status (Giovannini *et al,* 1995). Four patients

consumed **only** a mean of 80% or less than the EAR for energy (DH, 1991), but still

achieved acceptable morning plasma phenylalanine concentrations.

There was no significant relationship between energy intake and overall phenylalanine tolerance, although there is some evidence to suggest that patients who ate higher quantities of excess protein from free foods had higher energy intakes. However, Güttler showed that the administration of a protein free snack in a child with PKU had no impact on plasma phenylalanine concentrations (Güttler *et al,* 1969). This is supported by a further case study in an adult with PKU, in which a high carbohydrate snack was given late in the evening and did not prevent the nocturnal rise in phenylalanine concentration (Farquhar *et al,* 1985).

8.2.3 Rationalisation of allocation of fruit and vegetables in the PKU diet

Potatoes

These are popular phenylalanine exchanges and for years the quantity allowed per exchange has been underestimated. Phenylalanine analysis has been calculated indirectly from protein rather than by specific phenylalanine measurement. Although direct phenylalanine content is now available, there is no certainty of its accuracy. The phenylalanine content of potatoes varies according to age, variety, cooking method and measurement technique. Analyses also vary between different countries (Weetch *et al,* 1997; Bremer *et al,* 1996). In the potato study **(chapter 3)** which used phenylalanine exchanges based on direct phenylalanine measurement, portion size of potato increased by 33 to 220% according to the cooking method and the mean phenylalanine intake increased by **27** mg daily. It was, therefore, important to establish that this did not have any adverse effect on blood phenylalanine control. Potato phenylalanine exchanges based on direct phenylalanine measurement were tested daily over 5 weeks to try and detect any cumulative effect of the increase in

phenylalanine intake. **A** mean of two potato exchanges per day was eaten. This study demonstrated that it was safe to use the new potato exchanges as there was no significant changes in blood phenylalanine measurements in any of the patients. It is likely that the extra phenylalanine still provided a ratio lower to plasma phenylalanine pool size. Therefore the potato exchanges based on direct phenylalanine measurement have safely allowed significantly larger portion sizes of potato to be incorporated into the diet, a situation which is much appreciated by the patients.

Fruit and vegetables containing phenylalanine between 51-100 mg/100g

The results of this study **(chapter 4)** provide opportunity to rationalise and redefine the allocation of fruits and vegetables containing phenylalanine between 51-100 mg/100g in the diet. **As** the diet in PKU is so limited in variety, the ability to extend the range of unmeasured natural foods is welcome.

The lack of effect of additional phenylalanine from these fruit and vegetable sources was unexpected. Patients had a controlled increase in their median daily phenylalanine intake by 51 mg and 39 mg on the days of dietary assessment in part 2 and **3** of the study and neither had any impact on phenylalanine concentrations. For patients who entered part 3 of the study, their natural protein intake increased from a base of **46** to *86%* over allocated phenylalanine exchanges over the study period. There was no evidence that patients reduced the intake of other foods to compensate for the extra volume of food provided by these fruits and vegetables.

The reasons for the lack of impact of additional dietary phenylalanine is unclear, although there are a number of possible explanations:

1) The selected test amounts of h i t s and vegetables may not have big enough. However, this is unlikely as fruit and vegetables containing phenylalanine between 51-75 mg/100g e.g. bananas, mushrooms and raisins, were popular with the patients and a median daily portion size of 83g for the entire five week evaluation period is a useful test quantity. Many young children ate one banana a day and the highest quantity consumed was 200g daily. Some children might exceed this quantity and eat these foods to excess, but this applies equally to other fruits and vegetables that may contain phenylalanine just less than 50 mg/100g and which are already allowed freely. The median portion size for vegetables containing phenylalanine between 76-100 mg/100g was smaller at only 50g and these were taken only three times per week. However, the period of phenylalanine testing was extended by a further **2** weeks and one portion of these vegetables had to be eaten daily during blood sampling. The largest portion sizes of just over 100g were eaten by patients over the age of 10 years in whom a higher plasma phenylalanine of up to 700 μmol/l is allowed in accordance with MRC guidelines (MRC, 1993b).

2) Although these fruits and vegetables provided an increase in dietary phenylalanine, it may still not have been enough to alter phenylalanine concentrations as the daily phenylalanine is delivered in divided doses at and between mealtimes.

3) The non-starch polysaccharide content of the fruit and vegetables may have reduced their digestibility.

4) **A** concomitant increase in energy with the addition of the fruit and vegetables is unlikely to have had a major impact on protein utilisation. Energy only increased by a modest 68 kcal and 40 kcal daily for part two and three of the study, respectively.

It would therefore appear safe to incorporate fruits and vegetables tested in this study containing phenylalanine between 51-75 mg/100g into the diet without restriction. The evidence would also suggest that fruit and vegetables containing phenylalanine between 76-100 mg/100g could be incorporated without measurement. However, the portion size tested in the second part of this study was modest, and although there was no evidence that they had any impact on plasma phenylalanine concentrations, they should probably be restricted to one portion daily until further data is available.

In conclusion, the overall work on the phenylalanine content of fruit and vegetables and effect on plasma phenylalanine concentrations has provided evidence to enable the PKU diet to be more rationally based, but at the same time has increased free food choice and hence tolerability of the diet.

8.2.4 Effect of diet on feeding behaviour in young children with PKU

The study reported in **chapter 7** is the first to describe the extent of feeding problems in young children with PKU. Forty-seven per cent of mothers perceived their children to have

at least three feeding difficulties. Principal problems perceived and observed was slowness to feed, a poor appetite, a dislike of sweet foods and a limited variety of foods consumed. Parents perceived their children to have more gastrointestinal symptoms whilst it was observed that carers of PKU children resorted to more mealtime coercive strategies to persuade their children to eat, used less verbal encouragement at mealtimes and were more likely to feed their children in isolation. Although PKU children were lighter and smaller than control children, only two PKU children were stunted (height age more than 2 standard deviations below reference value). However, both of these children had at least one small parent, and neither had low energy intakes.

Energy intake

Although it was clear from carer's perception and observed video work that children with PKU had a poor appetite, they still achieved a median energy intake equivalent to the EAR. In most children with PKU, the protein substitute provided over 30% of the energy intake. This was through the use of XP Maxamaid (Scientific Hospital Supplies), which is supplemented with carbohydrate. **An** average daily toddler dose provided 370 kcal, and it is likely this suppressed appetite and so reduced energy intake from other foods. It is interesting to note that two children who took a protein substitute providing less than 20% of energy intake appeared to have less feeding difficulties. Carers were not generally aware of the amount of energy supplied by the protein substitute. Therefore, they may have had unrealistic expectations of how much food their children should eat and may have exacerbated feeding problems by coercing children to eat when they were not hungry.

Parental feeding strategies

It was clear that the carers of PKU children were more likely to cajole their children to eat, and to resort to bribery, and even threats. However, apparent poor food intake may not have been the only cause of this. Dietitians emphasise the importance of eating a set number of phenylalanine exchanges per day and carers may become preoccupied in ensuring all exchanges are eaten by their children. Repeated exchange refusal may lead carers to attempt to over-ride behaviour signals of satiety in their children. This in turn may help to nurture and sustain feeding problems and contribute to the increased incidence of negative feeding behaviour. Coercive mealtime management is known to make mealtimes unpleasant for children (Harris and Booth, **1987).**

Carers were less likely to sit with PKU children at mealtimes and give verbal encouragement and praise to persuade them to eat. In practice, carers had to prepare two different family meals, and they found it difficult to serve both simultaneously. **As** a result, children with PKU were frequently fed first and given their meals alone. However, lack of praise, unpleasant conversation about food and eating in isolation, can only have a negative effect on appetite and feeding.

Food preferences and dislikes

These were uncommon in the group of PKU children studied. Savoury foods were favoured (e.g. cucumber, tomato and crisps); food dislikes tended to be sweet (e.g. puddings, low-protein cakes and biscuits). It may be that the introduction of a bitter tasting protein substitute in early infancy influenced this preference. Furthermore, carers perceived their children with PKU to eat a limited range of foods even within the confines of their dietary

restriction. Some said they tended not to repeatedly offer food that had been initially refused. Food preference is, however, a function of exposure (Birch *et al,* 1982; Birch and Martin, 1982; Harris and Booth, 1987). If a child can be motivated by repeated social reinforcement to taste a food, then a preference for that food may be induced (Birch *et al,* 1987). It has been suggested by Birch and Martin that repeated experience with and exposure to a food up to 15 times will eventually induce a preference (Birch and Martin, 1982). This does, however, need careful interpretation. It should not be tried with foods to which there is a strong aversion, because this may induce loathing and exacerbate feeding problems.

Protein substitute

Particular problems were experienced with the administration of the protein substitutes. Based on L-amino acids, they are bitter tasting and produce hyperosmolar solutions when dissolved in water (XP Maxamaid osmolality **[*1:5*** dilution] 969 mosmol/kg). They then can be taken as a drink or **a** paste. Because of extreme difficulties in giving the protein substitute, a third of the parents, gave the majority once daily, often in the morning and usually in a liquid of less than *1:5* dilution. Carers reported constipation and/or abdominal pain in four out of five of these children. The same children were observed to have particularly poor appetites and were very slow feeders.

For all the PKU children, negative behaviour such as crying, screaming, deliberately spilling the drink and gagging were common when taking the protein substitute. Parents commented that they found administration of the protein substitute more tearful and stressful than giving natural food and one mother even stated that the time and effort in having to give the protein

substitute was ruining her family's life. Although the taste of the protein substitute is probably the main cause of these problems, there is scope to examine alternative ways of administering protein substitute in order to alleviate these difficulties.

This study, therefore, identified a number of feeding problems in young PKU children, some of which are likely to be precipitated by the protein substitute. Although the energy content of protein substitutes might have a beneficial role in helping to maintain an adequate energy intake in a restricted PKU diet, the protein substitute is probably suppressing appetite, particularly when administered before mealtimes.

Although the aetiology of feeding problems may be unclear, problem solving is important in PKU; otherwise these difficulties may lead to years of parental frustration and stress and may possibly impair nutritional status. Although an optimum dietary approach in young children with PKU is necessarily relatively rigid, an appreciation and awareness of the potential consequences of such diet therapy on feeding behaviour and growth should prove helpful in management and may lead to better plasma phenylalanine control in young children with PKU.

8.3 Potential drawbacks of individual studies

Chapter 2: Factors affecting the variation in plasma phenylalanine in patients with phenylketonuria.

Analysis of dietary phenylalanine intake

Due to lack of comprehensive phenylalanine analysis data, the phenylalanine intake in the first study may have been over-estimated. There were only three UK sources of

phenylalanine data: 1) the First Supplement to *'McCance and Widdowson's The Composition of Foods'* (Paul *et al,* 1980); *2)* the Leatherhead Food Research Association and the Laboratory of the Government Chemist (Weetch **e?***al,* 1997); and **3)** food manufacturers of special products. In total, these only provided phenylalanine analysis data for fruits, vegetables, some dairy products, low protein special foods, sauces and a limited number of cereals. For many composite foods e.g. vegetable nuggets, waffles, hash browns, potato shapes, phenylalanine analysis is unavailable, so it was estimated that 1g protein yielded 50 mg phenylalanine (Barnes, 1994). **As** many fruit and vegetable only yield 30–40 mg of phenylalanine per 1g of protein it is likely that phenylalanine based on these calculations was overestimated, particularly in younger children who eat many of these foods as part of their phenylalanine allowance.

Chapter 3: A prospective cross-over trial assessing the impact of new potato exchange allowance on blood phenylalanine concentrations in PKU.

Chapter 4: A prospective, cross-over trial investigating the effect of free use of fruits and vegetables containing intermediate amounts (51 – 100 mg/100g) of phenylalanine on control in PKU.

Open study design

These were both open studies. The researcher's enthusiasm for a specific treatment may have influenced the collection and interpretation of data. However, 1) the results were different from those anticipated by the researcher; 2) laboratory staff were blind to the treatment when analysing blood specimens; **3)** weighed food records were used so the researcher did not have to interpret portion sizes; and **4)** with the exception of routine blood results, all results were not given to parents or subjects until the end of the study.

Non-randomised trials

Neither of these two studies were randomised. The test period always followed the control period. There were three main reasons for this:

- the diet is very restrictive and it seemed insensitive either to reduce portion size or remove specific fruit and vegetables when they had previously been permitted freely.

- the trial period was always longer than the control period to ensure there was sufficient time for the extra phenylalanine taken to influence plasma phenylalanine concentrations.

- to help with compliance, as the second stage trial phase always permitted extra food. It may be expected that the plasma phenylalanine concentrations in the second stage of the trial may be higher due to study fatigue, but there is no evidence to indicate this.

Recruitment

This was difficult due to inability to eat the specified quantity of potatoes, fruit, and vegetable being studied.

Chapter 5: 24-hour plasma phenylalanine variability in PKU.

Blood sampling: venous sampling technique versus skin puncture

A combination of venous blood sampling and thumb skin punctures were incorporated into the protocol: hourly venous sampling during the day in hospital and **3** hourly thumb, heel or finger skin punctures overnight. Although it is widely presumed there is little difference in the results between thumb skin puncture and venous sampling techniques, there are no published data to support this. It is likely that the quality of skin puncture analysis is

influenced by operator skill, and in these studies all parents were trained and experienced at taking skin puncture blood specimens.

Subjects were studied for only 24 hours

It was considered unethical to ask subjects to repeat the 24-hour testing. It would also have been practically difficult, as subjects would have needed hospital admission for any study extension.

Chapter 6a: the effects of altered timing of administrations of protein substitute on variability in plasma phenylalanine concentrations.

Chapter 6b: the effects of frequent administration of protein substitute on plasma phenylalanine concentrations.

No washout time between protocols

This was for three reasons:

- Previous study work **(chapter 2 and 5)** suggested that adjusting timing of protein substitute had an immediate effect on plasma phenylalanine concentrations, probably because metabolism of the protein substitute is rapid.

- There was a 5 day period to adapt to taking the protein substitute before blood sampling. This was considered adequate to have counteracted any effects of a previous regimen.

- Washout time would have increased overall time taken to complete the study, which may have influenced recruitment and compliance.

Chapter 7: Abnormal feeding behaviours in PKU

Control group

Each child with PKU had only one matched control, so for outcome measures with wide individual variations it is difficult to draw definite conclusions. However, because it was only practical to study one matched control, these were matched for age, sex, housing and location, and each subject was studied in detail.

Video recording

There was a design problem of the video recording in that it was carried out in the home, and it was impossible to hide the camera. This may have acted as an added distraction for the child during the mealtime or caused carers to modify their feeding strategies. However, all the children became acquainted with the researcher before the mealtime, and had an opportunity to examine and play with the camera. In addition, this methodology was applied equally to both groups.

Dietary assessment

Three-day estimated food records, rather than weighed food intakes, were used to describe food intake. This was for two reasons: 1) only information about food types, patterns and a broad estimate of macronutrient intake was required; and **2)** to encourage compliance of the control group. However, many children's snacks were individually portioned, and weights quoted on the packaging helped with interpretation of portion sizes. The parents of PKU children continued to weigh all phenylalanine exchanges and protein substitute. All food records were checked with parents for any food omissions.

NB. In all studies a higher proportion of females to males were enrolled. This **is** a reflection of the under 10year old population of children attending the Birmingham Children's Hospital, which is predominantly female. However no gender difference has been noted in non-PKU children with blood phenylalanine concentrations, although adolescent males have been shown to have slightly higher phenylalanine concentrations than females (Gregory *et al,* **1986).**

8.4 Unanswered questions and future research

24 hour variability of plasma phenylalanine concentrations

The effect of wide 24-hour variability of plasma phenylalanine concentrations in PKU can only remain speculative. There is only tenuous evidence to link transient hypophenylalaninaemia with poor growth or neurological outcome. This can only be answered by long term, controlled prospective trials where the diet is manipulated to ensure transient hypophenylalaninaemia for 50% of the day.

Optimum timing for administration of protein substitute

The results of this study suggest that the optimum protein substitute administration regimen to achieve a stable blood phenylalanine profile over 24 hours, is to give small doses at least every 4 hours day and night. This is impractical. Some children under **8** years of age have great difficulty in keeping awake after 21.00hours. Alternative approaches need to be explored and investigated. Enteral feeding of protein substitute represents an alternative method. However, this is expensive, invasive and difficult for both patients and parents. Perhaps manufacturers should explore the concept of developing protein substitutes to release amino acids at different and slower rates. This may be afforded by the use of

thermally modified starches, which are currently being investigated as potential sustained release agents for oral-drug delivery (Remon *et al,* 1996). Realisation of this approach could lead to a regimen of fewer protein substitute doses over 24 hours. This would not only be more acceptable and convenient to patients, but also would reduce plasma phenylalanine variability and may increase phenylalanine tolerance. Further study is warranted.

Optimum daily dosage of protein substitute

The 24-hour plasma phenylalanine variability results reported in this thesis are different from those reported by van Spronsen (van Spronsen *et al,* 1993). This may relate to the higher daily dose of protein substitute used in the UK. As protein substitute suppresses plasma phenylalanine concentrations, higher total daily dosage may increase difference in phenylalanine variability seen i.e. the more protein substitute given, the bigger the fluctuations in phenylalanine concentrations. The most appropriate dose of protein substitute in PKU as not been determined. Although it is likely to be age and weight dependent, it should perhaps vary according to the severity of PKU. Carefully controlled trials are urgently needed to determine the optimum daily dose of protein substitute in PKU.

Practical administration and composition of protein substitute

In the feeding problem study, it is likely that the energy content, timing and administration of protein substitute in the form of a large drink inhibited appetite, contributed to the gastrointestinal symptoms and caused some of the feeding difficulties observed.

Since completing this study, it is now our normal practice to give the protein substitute as a paste, usually in 3–4 doses daily. **Only** a small volume of the product is taken at one time

and there appears to be a lower incidence of gastrointestinal symptoms. Difficulties with protein substitute administration also appear to have decreased but appropriate investigation is needed before any conclusions can be drawn. The effect of energy content of protein substitute, and it's method and timing of administration on appetite requires further controlled study.

8.5 Conclusion

Although the structure of the PKU diet used in the UK is relatively relaxed and flexible, this work highlights the many practical problems and adverse effects on feeding behaviour that such a rigid diet can cause. The need to maintain low blood phenylalanine concentrations in PKU is unequivocal. However, this work has demonstrated the wide variability in blood phenylalanine concentrations over **24** hours and the difficulties in interpreting single blood phenylalanine estimations. Plasma phenylalanine variability can be reduced to almost normal physiological changes by regular and more frequent administration of protein substitute day and night. It has also been demonstrated that it is better to use food phenylalanine content to estimate intake rather than protein content as the latter over-estimates phenylalanine intake. This work has enabled the phenylalanine exchange and free system to be rationalised in PKU. This in turn has led to further relaxation of the diet, and will hopefully help make the diet easier. Overall, this work has provided further insight into the effects of the different dietary components on blood phenylalanine concentrations; and has demonstrated the many problems faced by parents and children with PKU.

Appendix 1

FEEDING ASSESSMENT

FORM

Name ....

d.o.b. ....

Date ....

Study Patient Number ....

Weight ....

Height ....

Mid-arm circumference ....

1. Does your child have any of the following feeding problems?

           a) a poor appetite

           b) eats a limited variety of foods

           c) is slow to feed

           d) cannot chew food

           e) no problems

           f) other

             please specify _____

             _____

2. Does your child have any of the following problems?

           a) vomiting

           b) constipation

           c) diarrhoea

           d) abdominal pain and colic

Management

3. Feeding position at home?

      lap   standing

      baby bouncer   cot

      high chair   table/chair

      setee/armchaire   other

                            please specify _____

4. In what room do you feed your child?

      living room   playroom

      dining room   bedroom

      kitchen   other

5. Who mainly feeds or supervises feeding?

parents ☐	child's friend ☐
brothers/sisters ☐	neighbour ☐
all family ☐	grandparents ☐
by self ☐	other ☐

Please specify _____

7. What are the usual times of the (a) meals   (b) drinks
(not protein substitute)

	(a)	(b)
Breakfast	....	....
Mid-morning	....	....
Midday	....	....
Mid-afternoon	....	....
Evening Meal	....	....
Bedtime snack	....	....

8. Are feeding times: (put a mark on the scale showing how you feel).

a)   Relaxed   Stressful

|_____|_____|_____|_____|_____|
 1   2   3   4   5

b)   Noisy   Quiet

|_____|_____|_____|_____|_____|
 1   2   3   4   5

c)   Unrushed   Hectic

|_____|_____|_____|_____|_____|
 1   2   3   4   5

d) Tearful for parents   Happy for parents

|____|____|____|____|
1   2   3   4   5

e) Happy for child   Tearful for child

|____|____|____|____|
1   2   3   4   5

.

9. Is your child's appetite:

Poor   Good

|____|____|____|____|
1   2   3   4   5

10. Do you think your child eats enough?

Yes   No

|____|____|____|____|
1   2   3   4   5

11. Is your child difficult to feed?

Not difficult   Difficult

|____|____|____|____|
1   2   3   4   5

Feeding behaviour/appetite

12. Does the amount of food taken by your child fluctuate from day to day?

Yes   ☐
No   ☐
Sometimes   ☐
Don't know   ☐

13. Does your child accept foods one day but reject them on another?

Yes ☐

No ☐

Sometimes ☐

Don't know ☐

14. Does your child accept new foods?

Yes ☐

No ☐

Sometimes ☐

Don't know ☐

15. Please indicate the average duration of time to eat the evening meal/tea.

0 - 10 minutes ☐

10 - 20 minutes ☐

20 - 30 minutes ☐

30 - 60 minutes ☐

over 60 minutes ☐

16. Duration of time to eat snack e.g. biscuit?

0 - 10 minutes ☐

10 - 20 minutes ☐

20 - 30 minutes ☐

30 - 60 minutes ☐

over 60 minutes ☐

17. Does your child exhibit any of the following when given food?

	No	Yes	Each meal	Once a day	Once a week
a) Throws food/pushes food away					
b) Spits food					
c) Chews food, but will not swallow					
d) Turns head away repeatedly					
e) Closes mouth when offered food					
f) Knocks spoon away					
g) Cries/screams at the beginning of the feeds					
h) Cries/screams at the end of the feeds					
i) Vomits after or during meal					
j) Dribbles food out of mouth					

If yes, how often? heads the last three columns.

18. If your child doesn't finish a course or part of a meal, what do you do?

Take it away ☐
Attempt to make child eat food ☐
Distract the child to eat ☐
Offer next course ☐
Offer child reward for eating ☐
Offer another food/meal ☐

19. If your child does not eat a phenylalanine exchange or part of an exchange, what do you do?

Leave the exchange but not replace it ☐
Leave the exchange but try and make it up with something else later in the day ☐
Exchange it for something else in the same course ☐
Distract the child to eat it ☐
Offer the child an award ☐
Exchange it for another exchange in the pudding/sweet course ☐

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20. If your child is a messy eater, does it bother you?

Yes ☐
No ☐
Don't know ☐

21. How many people does it take to feed the child?

Feeds self ☐   Three ☐
One ☐   Other ☐
Two ☐

22. Do you need to distract your child when eating?

Yes ☐   No ☐

If the answer is yes, please specify type of distraction.

Television ☐   Games of Aeroplanes ☐
Toys ☐   Other children playing ☐
Reading ☐   Other, please specify ☐
Singing ☐   _____

23. What type of food is offered?

	Does Eat	Can Eat	Never Tried	Can't/ Won't Eat	What happens when they refuse?
Stage 1 baby food					
Adult puree food					
Stage 2 baby food					
Adult mashed food					
Finger foods					
Normal adult consistency					
Fluids only					

24. Current feeding skills

Spoon fed by parent	☐
Finger foods fed by parent	☐
Feeds self with fingers	☐
Feeds self with spoon	☐
Feeds self with fork	☐
Uses knife	☐
	☐

Drinks from bottle	☐
Drinks from cup/glass	☐
Drinks from straw	☐
Pours own drink	☐
Prepares own snacks	☐
Has child ever self-fed?	☐
Other	☐

25. Which of the following foods are currently refused?

Milk substitute	☐
Soups	☐
Vegetables	☐
Bread/chapatti (LP)	☐
Fruit	☐
Crisps	☐
LP Choc	☐

Sweets/Choc (ordinary)	☐
Cakes/biscuits (LP)	☐
Potatoes/rice/spaghetti	☐
Breakfast cereal	☐
Puddings	☐
Squash/lemonade	☐
Other	☐

Non-PKU

Meat	☐
Fish	☐
Eggs	☐
Cheese	☐
Milk	☐

State reason for refusal of foods.

26. <u>Temperature</u> : Child's reaction to:

Hot food	likes/dislikes
Cold food (ice lollies)	likes/dislikes
Warm food	likes/dislikes

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27. <u>Flavour</u>: Child's reaction to:

Sour	likes/dislikes
Salty	likes/dislikes
Savoury	likes/dislikes
Sweet	likes/dislikes
Highly flavoured	likes/dislikes

28. <u>Food intolerance</u>
   Please list -

29. <u>Vitamin supplements</u>
   Please list -

30. <u>Dietary supplements</u>

   Glucose polymer
   Hycal/Fortical
   Other

   Quantity per day ....

<u>Comments</u>: Do you have any other problems with feeding your child that are not
covered here?

Name

Sample daily food intake
Breakfast

Mid-morning

Midday

Mid-afternoon

Evening meal

Bedtime

Appendix 2

PROTEIN SUBSTITUTE

QUESTIONNAIRE

Name ....

d.o.b. ....

Date ....

Study Patient Number ....

1. Which protein substitute does your child take?

a) XP Maxamaid
b) Aminogran food supplement
c) XP Analog + Aminogran food supplement
d) Phlexy 10 drink mix
e) Other
   Please specify _____

2. Does you child take all of the protein substitute?

a) Every day
b) Most days
c) Some days
d) Rarely drinks all of it
g) Never

3. How easy is it to get your child to drink the protein substitute?

a) Very easy
b) Child will drink it, but with persuasion
c) Can be difficult some of the time
d) Difficult all of the time

4. If your child leaves some of the protein substitute, how much is usually left?

a) Up to 10%
b) 11 - 30%
c) 31 - 50%
d) 51 - 75%
e) over 75%
f) only bits left in the bottom of the cup/
   bottle/beaker

5. Who mainly supervises the giving of the protein substitute?

Mother		Friend	
Father		Home helper	
Grandparent		Child minder	
Sister/brother		Other	

Please specify _____

6. How is the protein substitute taken?

a) as a drink 0 - 100 ml

b) as a drink 101 - 200 ml

c) as a drink 201 - 300 ml

d) as a drink 301 - 400 ml

e) as a drink over 400 ml

g) other - please specify:

7. How frequently is the protein substitute taken each day?

Specify Times

a) 3 times daily

b) twice daily

c) once daily

d) continuously throughout day

e) continuously throughout day
   and night

8. From what type of container is the protein substitute taken?

bottle

covered cup

covered cup with straw

open cup with straw

other - please specify _____

9. In what room do you feed your child?

Living Room   Playroom

Dining Room   Bedroom

Kitchen   Other

                     Please specify _____

10. Does the child usually take the protein substitute when other people are present?

Always by self   Grandparents

With mother   Brother/sisters

With father   Friends

Both parents   Different members of the family together

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11. Is giving the protein substitute (put a mark on the scale showing how you feel)

a)   Relaxed   Stressful

|___|___|___|___|
1   2   3   4   5

b)   Noisy   Quiet

|___|___|___|___|
1   2   3   4   5

c)   Unrushed   Hectic

|___|___|___|___|
1   2   3   4   5

d)   Tearful for parents   Happy for parents

|___|___|___|___|
1   2   3   4   5

e)   Happy for child   Tearful for child

|___|___|___|___|
1   2   3   4   5
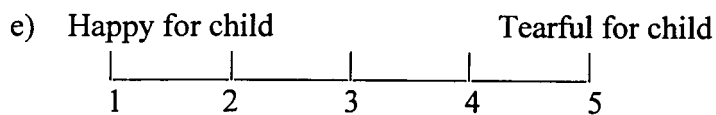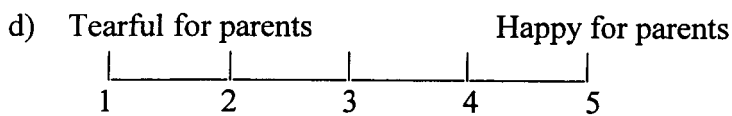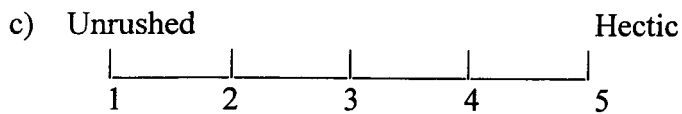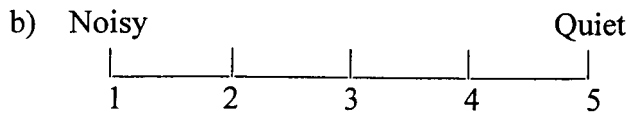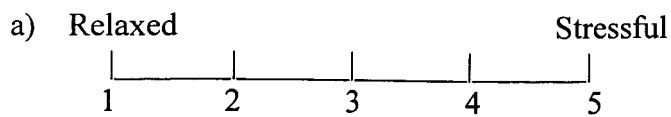
12. How many different protein substitutes has your child tried (excluding the baby protein substitutes)?

a) 1 ☐
b) 2 ☐
c) 3 ☐
d) 4 ☐
e) 5 ☐
f) 6 ☐
g) Other ☐
Please specify _____

13. Does your child accept new protein substitute readily?

a) Yes ☐
b) No ☐
c) Depends on taste ☐
d) Other ☐
   Please specify _____

14. Please indicate the average duration to drink the one portion of protein substitute.

0 - 10 minutes ☐
11 - 20 minutes ☐
21 - 30 minutes ☐
31 - 40 minutes ☐
40 - 60 minutes ☐
Over 60 minutes ☐
Don't know ☐

15. Please indicate the average duration of time to drink the total day's supply of protein substitute.

0 - 10 minutes ☐
11 - 20 minutes ☐
21 - 30 minutes ☐
31 - 40 minutes ☐
40 - 60 minutes ☐
61 - 120 minutes ☐
Over 2 hours ☐
Drinks all day ☐
Don't know ☐
Other ☐

16. Does your child exhibit any of the following when given the protein substitute?

	No	Yes	Each feed	Once a day	2/3 times a week
a) Throws cup/bottle pushes cup/bottle away					
b) Spits out protein substitute					
c) Holds protein substitute in mouth, but will not swallow					
d) Turns head away repeatedly					
e) Closes mouth when it is offered					
f) Cries/screams at the beginning of the protein substitute					
g) Cries/screams at the end of the protein substitute					
h) Deliberately spills some of the protein substitute					
i) Dribbles protein substitute out of mouth					

17. If your child does not finish the protein substitute what do you do?

Take it away

Attempt to make the child drink it

Distract the child to drink it

Offer the child a reward

Remove other privileges

A combination of the above each feed

Other

18. How many people does it take to persuade the child to drink the protein substitute?

Feeds self

One

Two

Three

Other

Please specify _____

19. If you distract your child when drinking the protein substitute, what do you do?

TV   Play games

Toys   Other children playing

Reading   Other

Singing   Please specify_____

20. Do you add a calorie supplement to the protein substitute?

Yes

No

Sometimes

21. If answered yes, please specify:

Type _____   Amount _____

22. What do you think will happen if your child does not drink the protein substitute?

a) Nothing ☐
b) Will not grow ☐
c) Will become unwell ☐
d) Does not get vitamins/minerals he needs ☐
e) Blood phenylalanine levels will go low ☐
f) Will not gain weight ☐
g) Blood phenylalanine levels will go high ☐

Appendix 3

Video Analysis Category System
(Adapted from Thomas and Harris 1993)

1. Meal duration.

2. Number of mouthfuls during meal.

Child feeding behaviour during the mealtime

1. Number of times child turns head away when offered food by parent.

2. Number of times child closes mouth when offered food.

3. Number of times child is redirected from the task of eating

4. Number of times child redirects self to the task of eating.

5. Number of positive vocalizations from child.

6. Number of negative vocalizations from child.

7. Does child spit food during meal? Yes/ No.

8. Does child feed self without prompting from parent at start of meal? Yes/No.

Parental intervention during the meal

1. Number of times parent offers food to child.

2. Number of vocalizations made by the parent:

−   feeding directed vocalization
−   prompting or redirecting child to task
−   bribing
−   praising
−   threatening
−   distracting

3   Is there any use of distracter items by the parent during the meal? Yes/No

4   Parental mealtime strategy if child does not finish course/meal:

−   take it away? Yes/No
−   attempt to make child eat food? Yes/No

- distract the child to eat? Yes/No
- offer next course? Yes/No
- offer child reward for eating? Yes/No

Definitions *of* categories used in video analysis

1 Meal duration. Time taken from when the plate is placed before the child to when the plate is taken away from the child

2 One mouthful. When child or parent places food in child's mouth. This does not include nibbling food and the food should be enough for child to chew.

Definition of child behaviour

1. **Child turns head away when proffered food by parent.** Child is seen to turn head away from the food being directly offered by the parent usually on a spoon or fork.

2. **Child closes mouth when proffered food by parent.** Child is seen to close mouth when the parent directly offers the food usually on a spoon.

3. **Child is redirected away from the task of eating; child is seen to turn away from the task of eating or drinking and to concentrate on some other activity.** This includes turning their head or body away from the plate, getting out of their chair or being distracted by other objects (e.g. by toys or the television) for a period of ten seconds after the last mouthful is finished. This movement should be self-induced and not as a result of vocalization from the mother.

4. **Child redirects from the task of eating.** This is when the child is seen to open their mouth and let the food or drink drop from their mouth or remove the food from their mouth forcibly with their tongue.

5. **Feeding/drinking without prompting from parent.** This is when the child is seen to start eating/drinking without being told by a parent.

Definition of parental non-verbal intervention

1. **Parent proffers food to child. A** direct attempt to offer food to the child, usually by placing food on a spoon or fork and lifting it up to the child's mouth or by putting a cup next to the mouth.

2. **Does the parent use a distracter item during the meal?** This could be anything from giving a toy, turning the television on during the meal.

3. **Parental mealtime strategy if child does not finish meal.** Defined when child indicates that they have had enough to eat by some form of verbalization or action having not completed the meal.

Definitions of parent/child vocalizations

The end of one vocalization is defined by a natural pause in the speech of the parent or child.

1. **Positive vocalizations from the child.** Positive speech from the child about the food without prompting from the parent e.g. a request for some more food or verbalization from the child illustrating their like for something they are eating.

2. **Negative vocalization from the child.** Negative speech from the child about food without prompting from the parent e.g. verbalization from the child saying they dislike something they are eating, or saying they don't want to eat any more.

3. **Feeding directed verbalization from parent.** Speech from the parent associated with the food being eaten e.g. asking the child "is that nice?" or "is the food too hot?"

4. **Prompting or redirecting the child to the task.** Direct speech from the parent reminding or encouraging the child to eat, usually because they are showing an interest in something other than the food in front of them e.g. "eat some more please"

5. **Bribing.** Speech from the parent promising some reward in order to encourage the child to eat e.g. "if you eat the rest you can have.. ."

6. **Praising.** Positive speech from the parent towards the child to show them that they are pleased that they are eating e.g. "good girl" or "good boy."

7. **Threatening.** Speech from the parent implying a negative consequence if the child does not eat what the parent wishes them to e.g. "if you don't eat it.. .."

8. **Distracting.** Speech from the parent with no direct reference to the eating situation accompanied by the parent offering food to the child.

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