# Bone health in phenylketonuria, reviewing the evidence

## Introduction

There have been conflicting reports as to whether adults and children with phenylketonuria (PKU) have worse bone health compared to their peers. There is a lack of consensus on the extent and cause of bone abnormalities (if any) within the PKU population.

Literature reporting bone health have included cross sectional and cohort studies. Different methodologies have been used to investigate bone health and the correlation to variables such as blood phenylalanine (Phe) control and protein substitute adherence. Many of the studies have failed to assess nutritional status, physical activity, body composition, and lifestyle choices (smoking, alcohol intake) or genetic factors which all influence bone health. Some studies have used mouse models, the human studies have recruited both children and adults in small numbers, and subjects have followed different dietary regimens with different nutrient compositions. This makes it difficult to interpret the literature in order to counsel patients on bone health in day-to-day clinical practice.

Bone health in PKU has had renewed interest since the introduction of glycomacropeptide (GMP)-based protein substitutes as an alternative to amino acid (AA)-based protein substitutes for the dietary management of PKU. It has been hypothesised that protein substitutes based on GMP could provide benefit for bone health in PKU.

**The objective of this evidence summary is to assess the available evidence relating to PKU and bone health in order to investigate the hypothesis that GMP-based protein substitutes are beneficial for bone health.**

**The aims of this evidence summary are:**

1. define bone health in the general population, including how it is measured
2. review the current evidence on bone health status in PKU
3. collate the evidence available on factors affecting bone health in PKU including dietary management
4. examine the literature related to bone health and GMP in PKU.

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## Collaborators

**Vitaflo® dietitians in collaboration with:**

Professor Anita MacDonald OBE, BSc, PhD, Consultant Dietitian, Birmingham Children's Hospital, UK and Anne Daly MSc, RD, Senior metabolic dietitian, Birmingham Children's Hospital, UK.

**Reviewed by:**

Professor Nick Shaw, Consultant Paediatric Endocrinologist, Birmingham Children's Hospital, UK.

# Contents



## 1 Bone health essentials

The human skeleton is a mechanical structure, designed to provide protection, structure and support. It is made up from two types of bone as shown in **figure 1**:

**Figure 1: diagram of human bone**

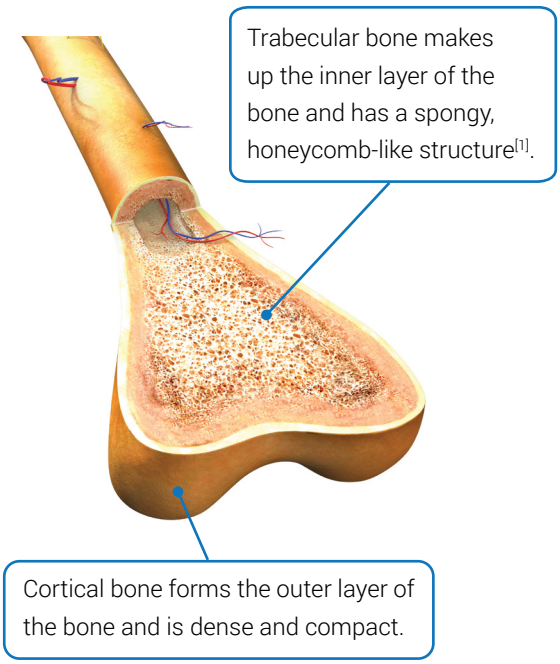
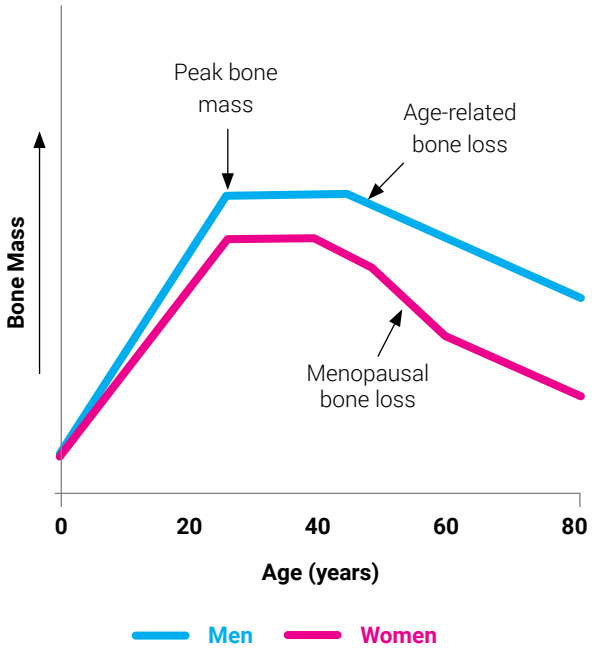



**Figure 2: accumulation of bone mass**



# 1 Bone health essentials

A child's skeleton is constantly changing in both size and composition. As the skeleton grows the bones are constantly being built and broken down; a process known as modelling and remodelling. Bone growth occurs in two ways; by increasing size and the accruing of bone minerals. In children these processes occur at different rates and times, however, by age 20-30 years the skeleton has reached its maximum, known as peak bone mass. Age-related bone loss then starts to occur where bone is removed faster than replaced[2]. This process is shown in **figure 2**.

A person's bone mass depends on the peak bone mass achieved and on the rate of loss later in life. Bone mineral density (BMD) is a measure of bone mass. Women experience an acceleration of bone loss around the time of menopause which lasts approximately 5-10 years[2, 3]. In children and adolescents who have not reached their peak bone mass, it is important to be able to assess if either bone growth or the build-up of bone minerals are altered, which would increase the risk of fragility fractures in childhood or later in life.

Low bone mass is associated with increased risk of osteoporosis and fracture[2]. Risk factors for low bone mass include:

- Unmodifiable factors: certain ethnicities, female gender, increasing age and family history of fracture[2]
- Modifiable factors: low body mass index (BMI), smoking, weight-bearing exercise, excess alcohol, vitamin D deficiency, low calcium intake, hormonal disorders and certain medications[2].

## Measuring Bone Health

Bone health can be measured in different ways including:

- **Bone blood parameters:** total plasma calcium, plasma phosphate, parathyroid hormone (PTH), 25-hydroxyvitamin D (25(OH)D), urinary calcium.
- **Bone blood turnover markers (BTM):** alkaline phosphatase (ALP), osteocalcin, type I collagen propeptides, deoxypyridinoline (DPD) cross-links of collagen, N-terminal and C-terminal cross-linked telopeptides.
- **Bone Mineral Density (BMD):** dual-energy X-ray absorptiometry (DXA), quantitative computed tomography (QCT), peripheral quantitative computed tomography (pQCT), quantitative ultrasound (QUS).
- **Diagnostic imaging:** radiographs (X-ray), radionuclide scans, magnetic resonance imaging (MRI).

## Bone blood turnover markers

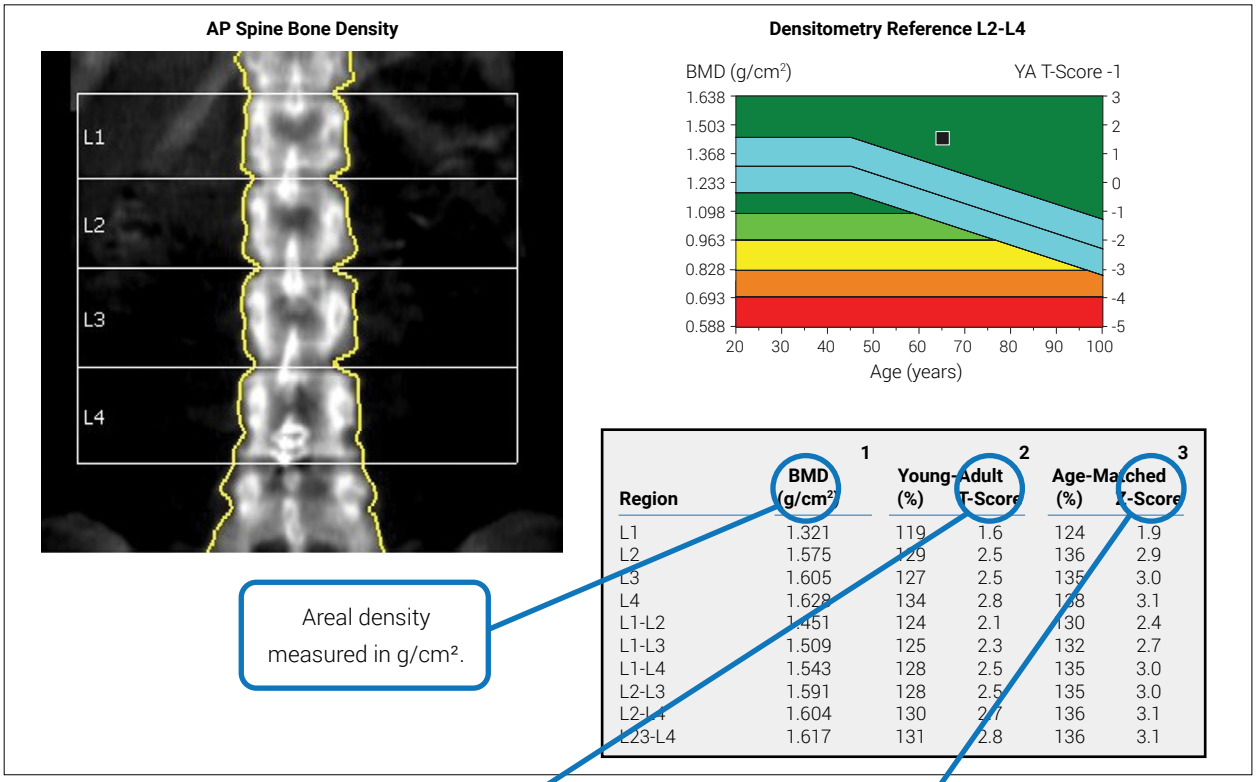
Bone turnover markers (BTM) involved in formation, resorption and regulation are released into the blood during bone remodelling. It has been advocated to use blood BTM in combination with the measurement of BMD to provide a more comprehensive clinical assessment of fracture and osteoporosis risk[4].

## Bone Mineral Density

BMD is a measure of the amount of calcium and other minerals (the bone mineral content) per square centimetre of bone. DXA is the most commonly used method to measure BMD. DXA measures a specific bone or bones, usually the spine L2-L4 (lumbar) and hip (femur) but can also be wrist (radius) and/or total (whole body). Reporting at different sites can alter the findings[1].

Spinal BMD is significant for trabecular bone, and femoral BMD for cortical bone. The density of measured bone is compared with an average index based on age, sex, and size to determine risk for fractures and the stage of osteoporosis (if any) in an individual[1, 5]. See **figure 3** for an example of a DXA scan report with explanation of the measurements which identify low BMD/osteopenia and osteoporosis. A specific image of the whole spine called the Lateral Vertebral Assessment (LVA) is particularly important and regarded as a standard measurement assessing skeletal fragility and undiagnosed spinal fractures.

**Figure 3: Example DXA scan report with measurements explained.**




### T-score

The number of standard deviations above or below the mean when the patient is compared to healthy 30-year-old adults of the same gender and, in some cases, ethnicity[3].

The World Health Organization diagnostic descriptors of T-score results are:

- Normal: −1.0 or higher
- Osteopenia/Low BMD: −1.0 to −2.5
- Osteoporosis: −2.5 or less
- Severe osteoporosis: -2.5 and below + fragility fracture

This is illustrated in the top right hand example graph with the black result box within the normal range for a 65 year old.

Because BMD declines with age, T-scores are consistently lower than Z-scores after about the age of 40 years, and the difference increases with age[6].

### Z-score

The number of standard deviations above or below the mean compared to patients of the same age and gender, and in some cases, ethnicity[3].

The International Society for Clinical Densitometry (ISCD) state: Z-scores of -2.0 or lower is defined as "below the expected range for age", and a Z-score above -2.0 is "within the expected range for age"[7].

Z-score are preferred when reporting BMD in females prior to menopause and in males younger than 50 years. The incidence of low BMD in the general population is 2%. Low BMD in children is defined as a BMD Z-score of less than -2. Osteoporosis cannot be diagnosed in children or men under 50 years based on BMD Z-score alone but must be coupled with a significant fracture history[3].

**NOTE:** Although DXA is the preferred method of measuring bone density, there are limitations when using this method in children. Children with reduced height compared to peers have an appropriate reduced bone mass. DXA overestimates bone density in a tall child while underestimating bone density in a short child; this may lead to misinterpretation of results. In children, the head constitutes a large portion of the total bone mass and is larger compared to body size. Therefore, it is important to exclude the head from DXA scans measuring total BMD in children, this adapted measurement is known as total body less head (TBLH).

## 2 Bone health in PKU

Bone health in PKU is affected by the same factors as the general population in addition to PKU specific factors. It has been suggested that genetics, and the necessity to follow a restrictive diet, could play significant roles. One of the first reports on abnormal bone health in PKU was by Feinberg and Fisch in 1962[8], which reported striations on long bones in neonates with PKU. In recent years there have been considerable advances in the dietary management for PKU and so it should be expected that updated outcomes for bone health should follow. Opinions remain divided and many clinical questions remain unanswered.

**There have been three systematic reviews published on bone health in PKU (Enns *et al*., 2010)[9], (Hansen and Ney, 2014)[10] and (Demirdas *et al*., 2015)[11]. Since Demirdas *et al*., 2015[11] systematic review and metanalysis was published there have been several more publications regarding bone health in PKU[12-20]. Findings from these reviews and the subsequent studies have been tabulated in appendix 1 to allow comparison. It is evident from these publications that low BMD in PKU is not universal.**

BMD has been the most investigated measure of bone health in PKU. BMD Z-scores are most commonly reported in studies of bone health in PKU because the population is relatively young and results from paediatric and adult subjects are combined[11]. However, no studies have reported BMD measurements in children as TBLH therefore caution is required when comparing with adult DXA Z-scores. Studies have also reported DXA Z-scores measured at different sites making comparison between studies difficult. A recent review provides more detail of the current techniques available to measure bone density and their potential limitations, it illustrates assessing bone health in children is challenging and measurements in isolation cannot provide a definitive diagnosis or conclusion[21].

All the publications in **appendix 1** that reported mean DXA Z-scores showed PKU patients to be within the expected range for age (above -2) according to ISCD official positions[7]. 5 publications compared DXA Z-scores with controls or reference populations. One conference report found no difference between patients and controls ($p<0.05$)[14]. Four publications reported BMD Z-scores as being lower in their PKU cohort [10, 11, 16, 19]. One recent publication compared adults only to the reference population and found mean BMD Z-scores were significantly lower for all skeletal sites except the radius[19]. Frequency of low BMD was observed in 1.6-5.5% with the maximum being observed at the spinal level, however, this is lower than described previously.

Statistical significance was not provided in the other three publications and authors were conflicted on the clinical significance of their findings[10, 11, 16].

Few studies have reported on bone mineral content (BMC) or bone turnover markers (BTM) in PKU. In the Demirdas *et al*., 2015[11] review authors reported the clinical implications of BMC in PKU were unknown. It stated that the results on BTM were ambiguous and consensus on the utility of BTM, including reliable methods of collection and reference ranges, should be established for further investigation. Geiger *et al*., 2016[13] found no abnormalities in BTM and Choukair *et al*., 2017[15] found normal BTM in the majority of their cohort.

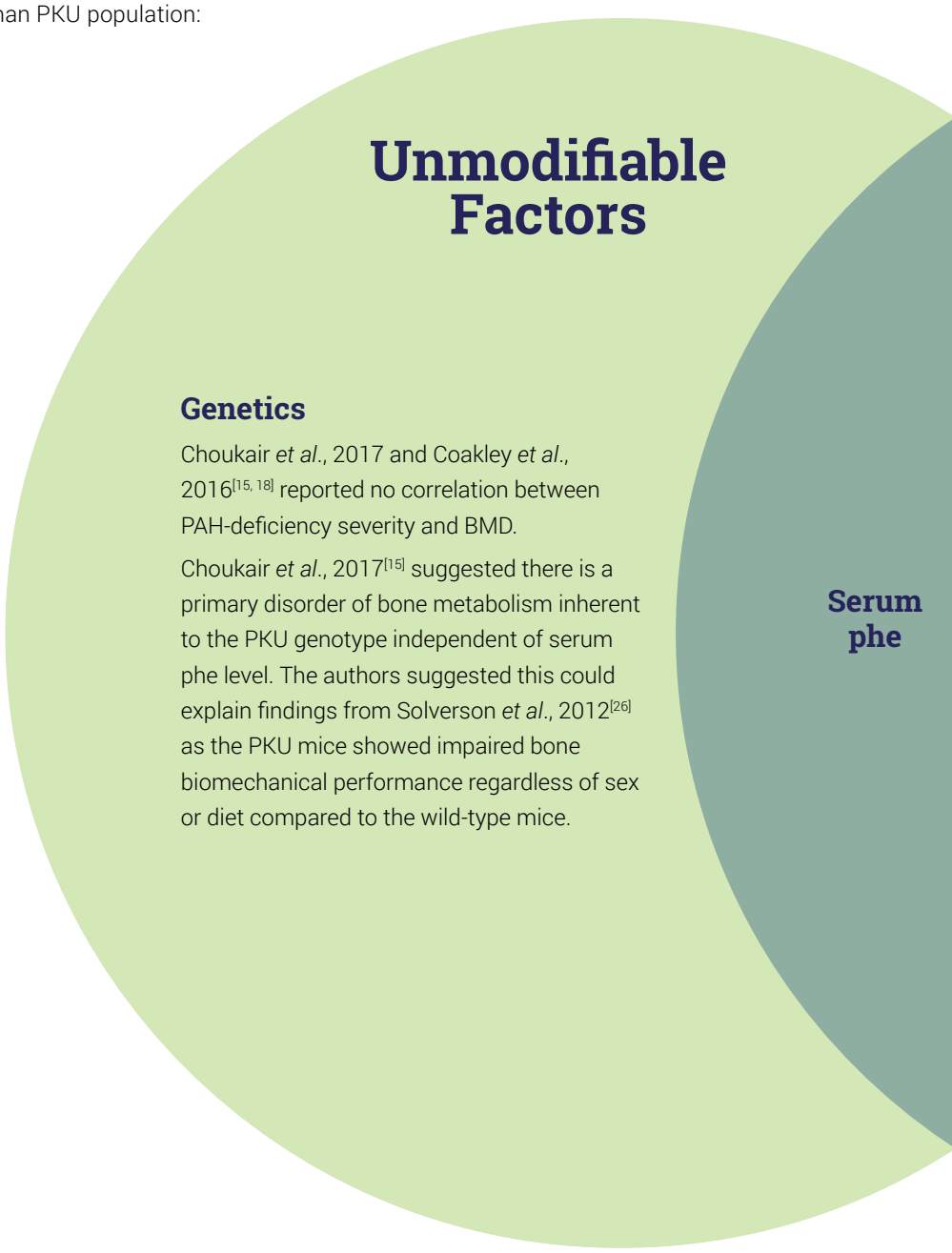
### Considerations

Recent studies on bone health in PKU, summarised in **appendix 1**, varied in study participants and methodologies including; BMD measured at different sites, wide age range, and use of sapropterin dihydrochloride (Kuvan®). Details on physical activity, body composition and fracture history are lacking. Many factors related to growth and development have been shown to influence BMD and peak bone mass in healthy children[22] with height particularly influencing BMD and BMC. While height of PKU children is statistically comparable to peers based on Z-scores[23, 24], other factors that need to be considered in PKU are weight bearing exercise, pubertal status and age at menarche, which are often unreported in study findings. Few studies report time spent exercising.

Nutritional intakes, genetic and lifestyle factors are known to influence bone development and health in the general population[2]. **Section 3** explores factors which have been identified to affect bone health in PKU. **Appendix 2** tabulates findings reported by studies included in **appendix 1** which investigated dietary associations.

## 3 Factors affecting bone health in PKU

**Section 2** summarised the evidence on bone health in PKU and demonstrated that the presence of impairment is contested. The likely cause of any bone impairment has been debated, whether it could be inherent to PKU or related to the dietary management[11, 15, 25]. Many factors affect bone health and skeletal development, some of which have been investigated in the human PKU population:



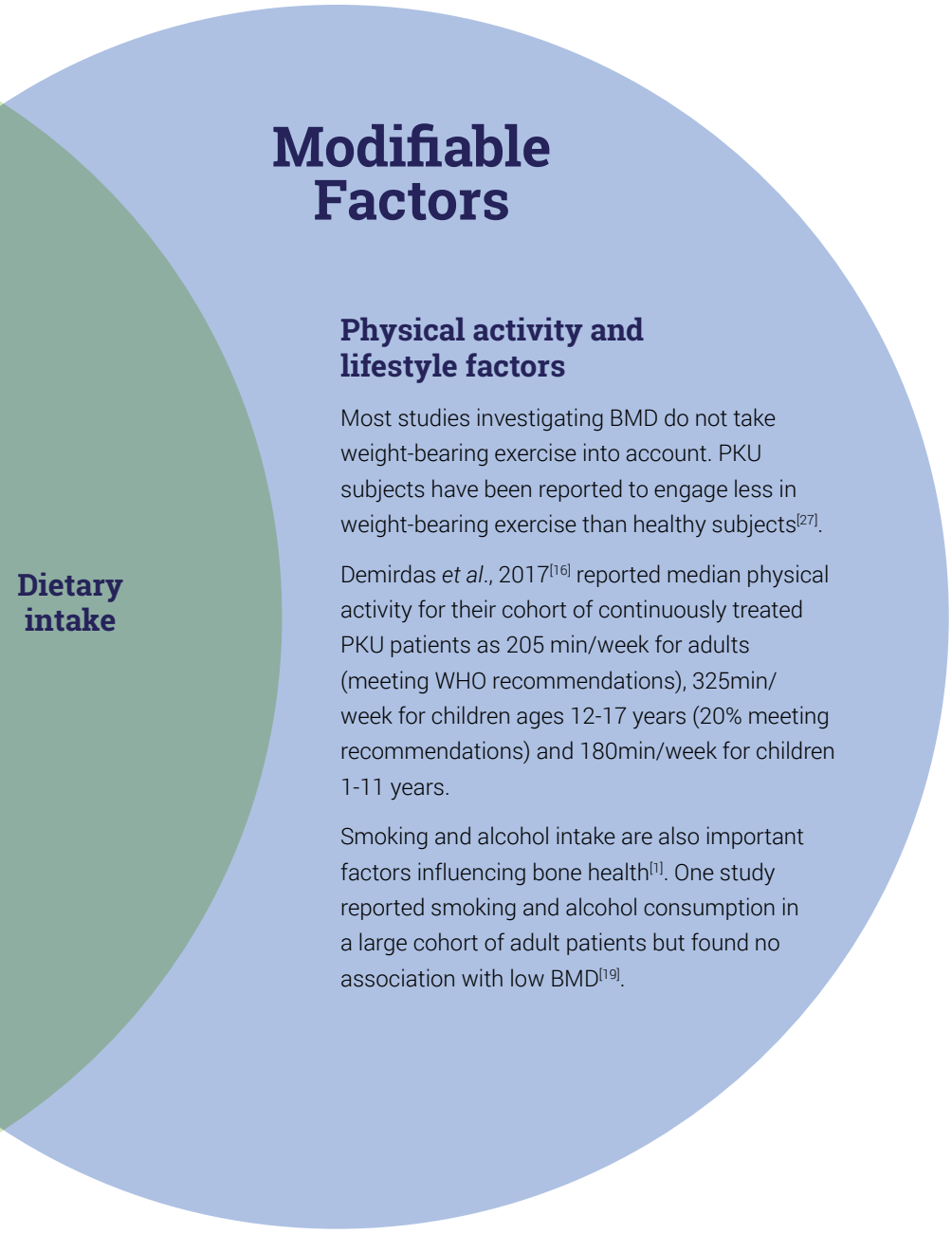

### Serum phe

Reviews by Hansen and Ney, 2014[10] and Demirdas *et al.*, 2015[11] concluded there were no correlations between serum phe and bone health in PKU. This finding has also been consistently reported in subsequent studies[13, 15, 18, 19].

### Dietary intake

**Appendix 2** tabulates the findings from the review and any subsequent studies which have investigated associations between dietary intake and outcomes of bone health. These primarily focused on protein, vitamin D, calcium, phosphate and magnesium.

Six out of the eight studies assessed for a correlation between bone health and nutrient intakes[11, 13, 17-19, 28]. One review reported no correlation between dietary intake and outcomes[11]. One study reported no correlation between calcium or




vitamin D supplementation and BMD[19]. Two out of the six studies reported BMD was significantly positively correlated with protein substitute intake in cohorts containing a combination of patients who were meeting minimum protein requirements (either synthetic or natural)[13, 18]. Two studies reported a non-significant negative correlation or no correlation between BMD and protein substitute intake, participants reported lifelong adherence to the PKU diet[17, 28]. The impact of overall protein status, including biological value of intact versus protein substitute and percent of total protein derived from protein substitute on bone were not considered by any of the studies.

Protein substitutes provide essential nutrients for bone health in PKU. Improvements in dietary management in recent years include stricter blood Phe target ranges, increased monitoring, improved access and continuing the diet for life[29-32]. Improvements in technologies to optimise taste and convenience of protein substitutes are linked to improved adherence and nutritional status[13, 15, 33, 34]. Therefore, assessment of bone health in individuals who have not received optimal nutritional management could explain an inaccurate representation of bone health in the PKU population.

## 4 GMP and bone health in PKU

Glycomacropeptide (GMP) is a macropeptide derived from a natural protein source. Un-modified it is an incomplete protein source. GMP is supplemented with the limiting, indispensable amino acids (apart from phenylalanine) in order to provide a viable alternative to AA-based protein substitutes for the dietary management of PKU. GMP-based protein substitutes have been shown to provide a suitable alternative to AA-based protein substitutes for growth, micronutrient status and metabolic control when used as the sole protein substitute or combined with other AA-based protein substitutes in adults and children with PKU[35-37]. It has been recommended that GMP-based protein substitutes should be introduced carefully and systematically in children[35].

Positive health benefits of GMP have been proposed including improvement of bone and gut health, prebiotic and anti-inflammatory properties and nitrogen retention[35, 38-43].

All the evidence on bone health in PKU summarised in **section 2** is based on research conducted on PKU patients taking AA-based protein substitutes. No published studies have measured bone health in patients whilst taking GMP-based protein substitutes. The long-term effects of GMP-AA-based protein substitutes on bone health in PKU are not established[31].

**Evidence available involving GMP-based protein substitutes and BMD in PKU:**

### (Solverson *et al.*, 2012)[26]

**Subjects:**

Wildtype (WT) and PKU mouse models (n = 21).

**Investigations:**

Mice consumed either a casein, AA, or GMP diet from weaning. DXA, 3-point bending testing and diaphyseal structure offemur. Total study length = 17 - 22 weeks.

**Findings:**

BMD was significantly lower in PKU mice compared to WT regardless of diet. No difference in BMD found between the diets in PKU mice.

In WT mice femur size and strength reduced in AA group compared to GMP and casein group.

**Considerations:**

Disease specific mouse models are produced with intensive brother-sister mating to produce mice with practically identical genomes in order to knock out specific genes more easily[44]. Differences seen in bone fragility between the inbred PKU and wildtype mice could be affected by genetic factors, this was supported by subsequent findings[15].

Activity levels were not assessed. It was acknowledged that neurological damage from Phe toxicity in PKU mice would likely have reduced physical activity levels and increased their risk of skeletal fragility. The differences in skeletal structure and development in mice compared to humans limit direct conclusion[13, 45].

### (Stroup *et al.*, 2017)[28]

**Subjects:**

8 early-treated PKU patients aged 16-35 years.

**Investigations:**

Two staged, crossover pilot study. Potential renal acid load (PRAL)* of protein substitute calculated. Food records and 24-hour urine collection after consuming low-phe diet in combination with high-PRAL* AA

**Findings:**

9 out of 10 AA-based protein substitutes had a 1.5–2.5-fold higher PRAL* than a GMP-AA-based protein substitute. A statistically significant increase in renal net acid excretion (RNAE) and calcium and magnesium urine losses were found in participants

**Considerations:**

Small cohort and short duration of GMP-based protein substitute exposure. DXA was taken at baseline when participants were taking AA-based protein substitutes and not repeated after GMP intake. High-

## Considerations

When reviewing the literature available on bone health and GMP-based protein substitutes it is important to consider that all published evidence relating to BMD measurements in PKU are based on dietary management with AA-based protein substitutes. Poor bone health reported in early, continuously and adequately treated patients with PKU is contested.

No studies have reported BMD or markers of bone health in patients taking GMP-based protein substitutes. Studies which suggest that GMP-based protein substitutes benefit bone in PKU attribute this to GMP-based protein substitutes providing a lower PRAL* value[17, 28]. A causal association between dietary acid load, measured with PRAL*, and osteoporotic bone disease is not supported by evidence in the general population[46, 47]. PRAL* calculation used to investigate protein substitutes in PKU is significantly influenced by mineral and electrolyte content of the product, particularly sodium content. Patients taking a high-sodium, low-PRAL* AA-based protein substitute were not included in the investigation. The correlation between BMD and PRAL* value of the protein substitutes, which would support the hypothesis that high PRAL* reduces BMD, was not reported in either publication. It was reported that the correlation between intake of high PRAL* protein substitutes and BMD measures did not reach statistical significance[17, 28].

* See **appendix 3** "What is potential renal acid load (PRAL) and how does it relate to PKU?"

-based, or low-PRAL* GMP-AA-based, protein substitutes for 1-3 weeks each. Patients taking a low-PRAL* AA-based protein substitute were excluded. DXA completed at baseline when taking usual AA-based protein substitute.

taking high-PRAL* AA-based protein substitutes, compared to those taking low-PRAL* GMP-AA based protein substitutes. Suggested that the cause of the increased skeletal fragility is associated with PRAL*.

sodium, low-PRAL* AA-based protein substitutes excluded. Correlation between PRAL and BMD was not published. PRAL* statistically significantly affected by sodium content of product.

**(Stroup *et al.*, 2017)[17]**

**Subjects:**

15 PKU patients aged 15-50 years.

**Investigations:**

DXA completed reflective of usual AA-based protein substitute. 3-day food record diaries. PRAL* of protein substitute calculated. 24-hour urine collection after 1-3 weeks of taking either high-PRAL* AA, or low-PRAL* GMP-based, protein substitutes.

**Findings:**

Males[6] had statistically significantly lower total body and femur BMD compared to females (no other BMD measurement reached statistical significance). Mean total femur DXA Z-score was negatively correlated with intakes of AA-based protein substitutes (p=0.048) but not spine or total body. No significant difference was found between male and female PRAL*, RNAE, or AA-based protein substitute intake (gPE/kg/day).

**Considerations:**

Small cohort and DXA not repeated after intake of GMP-based protein substitute. It was concluded that higher intakes of AA-based protein substitute with a higher PRAL* value results in low BMD in males, however, no significant difference between male and female PRAL* intake found. The correlation between mean PRAL* and BMD was not reported.

## 5 Discussion

Bone health in PKU is complex and recent studies have shown mean BMD Z-scores are within the normal range according to ISCD definitions[7, 10-18]. However, BMD is often lower compared to controls or reference populations, and the clinical significance of this is contested[10, 11, 16]. All research providing evidence of BMD, BMC and fractures have been based on patients who have taken AA-based protein substitutes. Few studies have investigated correlations between dietary intake and bone health. Those that have, linked improvement in bone health with adequate intakes of calcium and vitamin D, adherence to the phe-restricted diet and adherence to prescribed amounts of protein substitutes[13, 16, 18].

It has been suggested that GMP-based protein substitutes could be beneficial for bone health. Although the prospect of clinical benefit of GMP is appealing, more research is needed. Considering the complexity of genetic, clinical, nutritional and lifestyle factors which influence bone health, it is unlikely that changes in bone health could be attributed to a single dietary component such as GMP. No supporting BMD data in patients taking GMP-based protein substitute is currently available to allow any comparison to AA-protein substitutes; and the evidence available from Solverson et al. 2012[26], conducted on a mouse model, has limited application on informing clinical decisions for patients.

The report by Daly et al. 2016[12] represents baseline data of a 3-year clinical trial which will compare bone health, including TBLH, in children with PKU after consuming AA or GMP-based protein substitutes (PKU sphere®). Interim results suggest no significant difference between those taking GMP-based protein substitutes compared to AA-based protein substitutes for height, weight, BMI, percentage total fat lean mass BMC, bone mineral apparent density and TBLH[20]. However, this research is still yet to be fully analyzed and published which will provide further insight into the impact of GMP-based protein substitutes on bone health in PKU and allow comparison with AA-based protein substitutes in early and continuously treated children with PKU.

**Current practical recommendations to optimise bone health for individuals with PKU include[18, 31]:**

- **ensuring adequate intakes of calcium and vitamin D,**
- **regular weight-bearing exercise,**
- **optimisation of natural protein intake,**
- **promote adherence to prescribed amount of protein substitute.**

## 6 Conclusion

The objective of this evidence summary was to review the data available to investigate whether GMP-based protein substitutes are beneficial for bone health for individuals with PKU. More research is needed to conclusively determine whether GMP-based protein substitutes affect bone health in PKU. As stated previously, bone health is multifactorial and confounding factors need to be controlled for in future research. It is important to ensure future research is conducted with early treated individuals with PKU who have adequate nutritional intakes.

GMP-based protein substitutes offer an alternative choice for clinicians and patients, providing a different taste and mouthfeel which many patients find preferable to AA-based protein substitutes[34, 35, 48-50]. As a result, GMP-based protein substitutes could promote dietary adherence for individuals with PKU[34, 49, 51].

**Adherence to any protein substitute is likely to promote more optimal clinical outcomes for PKU patients, especially when the protein substitute is fortified with a comprehensive nutrient profile beneficial for bone health[18, 52-54].**

## 7 References


1. National Institutes of Health. Bone Basics. In: Osteoporosis and Related Bone Diseases National Resource Centre [Available from: https://www.bones.nih.gov/health-info/bone/bone-basics].
2. Kumar P CM. Clinical Medicine. Sixth Edition ed: Elsevier Saunders; 2008.
3. Cummings SR, Bates D, Black DM. Clinical use of bone densitometry: scientific review. Jama. 2002; 288(15): 1889-97.
4. Kuo T-R, Chen C-H. Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. Biomarker Research. 2017; 5(1): 18.
5. Lewiecki EM. Osteoporosis: clinical evaluation. Endotext [Internet]: MDText. com, Inc.; 2018.
6. Densitometry TISFC. 2015 ISCD Official Positions - Adults 2015 [Available from: http://www.iscd.org/official-positions/2015-iscd-official-positions-adult/].
7. Shuhart CR, Yeap SS, Anderson PA, Jankowski LG, Lewiecki EM, Morse LR, et al. Executive Summary of the 2019 ISCD Position Development Conference on Monitoring Treatment, DXA Cross-calibration and Least Significant Change, Spinal Cord Injury, Peri-prosthetic and Orthopedic Bone Health, Transgender Medicine and Pediatrics. Journal of Clinical Densitometry. 2019.
8. Feinberg S, Fisch R. Roentgenologic findings in growing long bones in phenylketonuria: Preliminary study. Radiology. 1962; 78(3): 394-8.
9. Enns G, Koch R, Brumm V, Blakely E, Suter R, Jurecki E. Suboptimal outcomes in patients with PKU treated early with diet alone: revisiting the evidence. Molecular genetics and metabolism. 2010; 101(2-3): 99-109.
10. Hansen KE, Ney D. A systematic review of bone mineral density and fractures in phenylketonuria. J Inherit Metab Dis. 2014; 37.
11. Demirdas S, Coakley KE, Bisschop PH, Hollak CE, Bosch AM, Singh RH. Bone health in phenylketonuria: a systematic review and meta-analysis. Orphanet Journal of Rare Diseases. 2015; 10(1): 17.
12. Daly A, Chahal S, Evans S, MacDonald A. Normal growth and bone mineral density reported in 38 PKU children taking conventional amino acid protein substitutes (P-124). J Inherit Metab Dis. 2016; 39 (Suppl 1): S93.
13. Geiger KE, Koeller DM, Harding CO, Huntington KL, Gillingham MB. Normal vitamin D levels and bone mineral density among children with inborn errors of metabolism consuming medical food– based diets. Nutr Res. 2016; 36(1): 101-8.
14. Leiva CA, Cornejo V, Bravo P. Bone mineral density in children, adolescents and young adults wtih phenylketonuria and hyperphenylalaninemia. J Inherit Metab Dis. 2016; 39 (Suppl 1): S250.
15. Choukair D, Kneppo C, Feneberg R, Schönau E, Lindner M, Kölker S, et al. Analysis of the functional muscle–bone unit of the forearm in patients with phenylketonuria by peripheral quantitative computed tomography. J Inherit Metab Dis. 2017; 40(2): 219-26.
16. Demirdas S, van Spronsen FJ, Hollak CEM, van der Lee JH, Bisschop PH, Vaz FM, et al. Micronutrients, Essential Fatty Acids and Bone Health in Phenylketonuria. Annals of Nutrition and Metabolism. 2017; 70(2): 111-21.
17. Stroup BM, Hansen KE, Krueger D, Binkley N, Ney DM. Sex differences in body composition and bone mineral density in phenylketonuria: A cross-sectional study. Molecular Genetics and Metabolism Reports. 2018; 15: 30-5.
18. Coakley KE, Douglas TD, Goodman M, Ramakrishnan U, Dobrowolski SF, Singh RH. Modeling correlates of low bone mineral density in patients with phenylalanine hydroxylase deficiency. J Inherit Metab Dis. 2016; 39(3): 363-72.
19. Lubout CMA, Blanco FA, Bartosiewicz K, Feillet F, Gizewska M, Hollak C, et al. Bone mineral density is within normal range in most adult PKU patients. J Inherit Metab Dis. 2019.
20. Daly A PA, Evans S, Rocha JC, Jackson R, MacDonald A. P-117. Body composition and bone mineral density in PKU children - interim results from a 3 year study. J Inherit Metab Dis. 2019; 42(S1): 2.
21. Shaw N, Crabtree N. Bone density in children: what are we measuring? Arch Dis Child. 2019; 104(11): 1108-11.
22. Maynard LM, Guo SS, Chumlea WC, Roche AF, Wisemandle WA, Zeller CM, et al. Total-body and regional bone mineral content and areal bone mineral density in children aged 8-18 y: the Fels Longitudinal Study. The American journal of clinical nutrition. 1998; 68(5): 1111-7.
23. Rocha JC, van Spronsen FJ, Almeida MF, Ramos E, Guimarães JT, Borges N. Early dietary treated patients with phenylketonuria can achieve normal growth and body composition. Molecular genetics and metabolism. 2013; 110: S40-S3.
24. Koura HM, Abdallah Ismail N, Kamel AF, Ahmed AM, Saad-Hussein A, Effat LK. A long-term study of bone mineral density in patients with phenylketonuria under diet therapy. Arch Med Sci. 2011; 7(3): 493-500.
25. de Groot MJ, Hoeksma M, van Rijn M, Slart RH, van Spronsen FJ. Relationships between lumbar bone mineral density and biochemical parameters in phenylketonuria patients. Molecular Genetics and Metabolism. 2012; 105(4): 566-70.
26. Solverson P, Murali SG, Litscher SJ, Blank RD, Ney DM. Low bone strength is a manifestation of phenylketonuria in mice and is attenuated by a glycomacropeptide diet. PLoS One. 2012; 7(9): e45165.

## 7 References


27. von Berlepsch J, Feldmann R, Koletzko B, editors. Determinants of obesity risk in adult patients with phenylketonuria. J Inherit Metab Dis; 2008: Springer Van Godewijckstraat 30, 3311 Gz Dordrecht, Netherlands.
28. Stroup BM, Sawin EA, Murali SG, Binkley N, Hansen KE, Ney DM. Amino acid medical foods provide a high dietary acid load and increase urinary excretion of renal net acid, calcium, and magnesium compared with glycomacropeptide medical foods in phenylketonuria. Journal of nutrition and metabolism. 2017; 2017.
29. Hollak CE, Lachmann R. Inherited Metabolic Disease in Adults: A Clinical Guide: Oxford University Press; 2016.
30. Pena MJ, de Almeida MF, van Dam E, Ahring K, Belanger-Quintana A, Dokoupil K, et al. Protein substitutes for phenylketonuria in Europe: access and nutritional composition. Eur J Clin Nutr. 2016.
31. van Wegberg AMJ, MacDonald A, Ahring K, Bélanger-Quintana A, Blau N, Bosch AM, et al. The complete European guidelines on phenylketonuria: diagnosis and treatment. Orphanet Journal of Rare Diseases. 2017; 12(1): 162.
32. Singh RH, Cunningham AC, Mofidi S, Douglas TD, Frazier DM, Hook DG, et al. Updated, web-based nutrition management guideline for PKU: An evidence and consensus based approach. Molecular genetics and metabolism. 2016; 118(2): 72-83.
33. Gokmen-Ozel H, MacDonald A, Daly A, Hall K, Ryder L, Chakrapani A. Long-term efficacy of 'ready-to-drink' protein substitute in phenylketonuria. J Hum Nutr Diet. 2009; 22(5): 422-7.
34. van Calcar SC, MacLeod EL, Gleason ST, Etzel MR, Clayton MK, Wolff JA, et al. Improved nutritional management of phenylketonuria by using a diet containing glycomacropeptide compared with amino acids. The American Journal of Clinical Nutrition. 2009; 89(4): 1068-77.
35. Daly A, Evans S, Chahal S, Santra S, Pinto A, Jackson R, et al. Glycomacropeptide: long-term use and impact on blood phenylalanine, growth and nutritional status in children with PKU. Orphanet Journal of Rare Diseases. 2019; 14(1): 44.
36. Pena MJ, Pinto, A., Daly, A., MacDonald, A., Azevedo, L., Rocha, J.C. and Borges, N. The Use of Glycomacropeptide in Patients with Phenylketonuria: A Systematic Review and Meta-Analysis. Nutrients. 2018; 10(11).
37. Pinto A. Nutritional status in patients with phenylketonuria using glycomacropeptide as their major protein source. Eur J Clin Nutr. 2017; 71.
38. van Calcar SC, Ney DM. Food products made with glycomacropeptide, a low-phenylalanine whey protein, provide a new alternative to amino Acid-based medical foods for nutrition management of phenylketonuria. J Acad Nutr Diet. 2012; 112(8): 1201-10.
39. Ney DM, Blank RD, Hansen KE. Advances in the nutritional and pharmacological management of phenylketonuria. Current Opinion in Clinical Nutrition and Metabolic Care. 2014; 17(1): 61-8.
40. Ahring KK, Lund AM, Jensen E, Jensen TG, Brøndum-Nielsen K, Pedersen M, et al. Comparison of Glycomacropeptide with Phenylalanine Free-Synthetic Amino Acids in Test Meals to PKU Patients: No Significant Differences in Biomarkers, Including Plasma Phe Levels. Journal of Nutrition and Metabolism. 2018; 2018.
41. Ntemiri A, Chonchuir FN, O'Callaghan TF, Stanton C, Ross RP, O'Toole PW. Glycomacropeptide Sustains Microbiota Diversity and Promotes Specific Taxa in an Artificial Colon Model of Elderly Gut Microbiota. J Agric Food Chem. 2017; 65(8): 1836-46.
42. Sawin E, Aktas B, DeWolfe T, Stroup B, Murali S, Steele J, et al. Glycomacropeptide Shows Prebiotic and Immune Modulating Properties in Phenylketonuria and Wild Type Mice. The FASEB Journal. 2015; 29(1 Supplement).
43. Hvas CL, Dige A, Bendix M, Wernlund PG, Christensen LA, Dahlerup JF, et al. Casein glycomacropeptide for active distal ulcerative colitis: a randomized pilot study. European journal of clinical investigation. 2016; 46(6): 555-63.
44. Jilka RL. The relevance of mouse models for investigating age-related bone loss in humans. Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences. 2013; 68(10): 1209-17.
45. Perlman RL. Mouse models of human diseaseAn evolutionary perspective. Evolution, medicine, and public health. 2016; 2016(1): 170-6.
46. Fenton TR, Tough SC, Lyon AW, Eliasziw M, Hanley DA. Causal assessment of dietary acid load and bone disease: a systematic review & meta-analysis applying Hill's epidemiologic criteria for causality. Nutrition journal. 2011; 10(1): 41.
47. Cao JJ, Johnson LK, Hunt JR. A diet high in meat protein and potential renal acid load increases fractional calcium absorption and urinary calcium excretion without affecting markers of bone resorption or formation in postmenopausal women. J Nutr. 2011; 141(3): 391-7.
48. Lim K, van Calcar SC, Nelson KL, Gleason ST, Ney DM. Acceptable low-phenylalanine foods and beverages can be made with glycomacropeptide from cheese whey for individuals with PKU. Molecular genetics and metabolism. 2007; 92(1): 176-8.
49. Ney DM, Stroup BM, Clayton MK, Murali SG, Rice GM, Rohr F, et al. Glycomacropeptide for nutritional management of phenylketonuria: a randomized, controlled, crossover trial. The American Journal of Clinical Nutrition. 2016.

## 7 References


50. Proserpio C, Pagliarini E, Zuvadelli J, Paci S, Re Dionigi A, Banderali G, et al. Exploring Drivers of Liking of Low-Phenylalanine Products in Subjects with Phenyilketonuria Using Check-All-That-Apply Method. Nutrients. 2018; 10(9): 1179.
51. Ney DM, Gleason ST, van Calcar SC, MacLeod EL, Nelson KL, Etzel MR, et al. Nutritional management of PKU with glycomacropeptide from cheese whey. J Inherit Metab Dis. 2009; 32(1): 32-9.
52. Rohde C, von Teeffelen-Heithoff A, Thiele A, Arelin M, Mütze U, Kiener C, et al. PKU patients on a relaxed diet may be at risk for micronutrient deficiencies. European Journal of Clinical Nutrition. 2014; 68(1): 119-24.
53. Schulz B, Bremer HJ. Nutrient intake and food consumption of adolescents and young adults with phenylketonuria. Acta Paediatr Scand. 1995; 84(7): 743-8.
54. Hochuli M, Bollhalder S, Thierer C, Refardt J, Gerber P, Baumgartner M. Effects of Inadequate Amino Acid Mixture Intake on Nutrient Supply of Adult Patients with Phenylketonuria. Ann Nutr Metab. 2017; 71: 129-135.


## 8 Glossary of terms

**Bone Density or Bone Mineral Density (BMD):** The average concentration of mineral in a 2- or 3- dimensional image or defined section of bone. This term is also used to refer to results of all types of bone densitometry.

**Bone Mass:** The amount of bone tissue as the total of protein and mineral in the whole skeleton or in a particular segment of bone.

**Bone Mineral Content (BMC):** The amount of mineral measured in a defined section of bone. Total bone mineral content refers to the amount of mineral in the whole skeleton or in a particular segment of bone.

**Dual-energy X-ray absorptiometry (DXA):** A measure of the amount of calcium and other minerals per square centimetre of bone and used to assess mass and fracture risk.

**Osteopenia:** A term originating from the Working Group of the World Health Organisation to refer to T scores between -1.0 and -2.5.

**Osteoporosis:** Defined by the Working Group of the World Health Organisation as a bone density T-score at or below -2.5. A diagnosis of osteoporosis is also made based on a vertebral fracture confirmed by radiograph.

**Total bone less head (TBLH):** DXA measurement which has excluded the head, used in assessment of BMD in children.

# 9 Appendices

| Author, year, country | Study type | Outcomes investigated | No PKU patients | Mean/median BMD Z-score (g/cm²) |
|---|---|---|---|---|
| **Enns *et al.*, 2010[9], USA** | Systematic review. | Not reported. | Not reported. | Not reported. |
| **Hansen and Ney[10], 2014, USA** | Systematic review. | BMD Z-score, fractures. | 67<br>Mean age in studies on BMD:<br>9 ± 2 years,<br>11 ± 4 years,<br>9 ± 4 years. | Spine: - 0.10. |
| **Demirdas *et al.*, 2015[11], USA and Netherlands** | Systematic review and meta-analysis. | BMD Z-score, BTM, BMC, says fractures reported but then not assessed in outcomes. | 360<br>Age range: 11-57 years. | Total: - 0.45.<br>Lumbar: -0.70.<br>Femur: -0.96. |
| **Geiger *et al.*, 2016[13], USA** | Single centre, cross-sectional retrospective record review and prospective cross-sectional study in PKU patients. | Hip and lumbar BMD, dietary intakes, 25 hydroxyvitamin D2 and D3, iPTH, plasma calcium, ALP. | 88 IEM retrospective review.<br>20 PKU prospective study.<br>Age range: 8-20y. | 16 had normal BMD at both hip and lumbar.<br>2 had reduced BMD in the hip (-2.4 and -3.6).<br>1 had reduced lumbar BMD (-2.1).<br>None had reduced BMD at both hip and lumbar.<br>Mean Z-scores not reported. |
| **Leiva *et al.*, 2016[14], Chile** | Single centre, cross-sectional study (conference abstract). | Lumbar, femur and total BMD. | 16<br>Age range: 6-23 years. | Control: lumbar: -0.4, femur: 0.2, total: 0.5.<br>PKU: lumbar: -0.3, femur: -0.3, total: 0.2.<br>mHPA: lumbar: -0.05, femur: 0.65, total: 1.25. |
| **Daly *et al.*, 2016[12], UK** | Baseline visit of a prospective, longitudinal, parallel, controlled study (conference abstract). | Height, weight, total %body fat, BMD Z-score, whole body BMD Z-score. | 38<br>Age range: 5-16 years. | Males: BMD: -0.3 whole body: -0.4.<br>Females: BMD: 0.3 whole body: -0.75. |
| **Demirdas *et al.*, 2017[16], Netherlands** | Multi-centre, prospective, cross-sectional study. | Dietary intake, blood micronutrient concentrations, fatty acid status, physical activity, fracture history, BMD Z-score. | 60<br>Age range: 1-39 years. | Lumbar: -0.1.<br>Femur: -0.45.<br>Hip: -0.3. |
| **Coakley *et al.*, 2016[18], USA** | Single centre, prospective, cross-sectional study. | Anthropometry, BMD Z-score, BMC, dietary intake, blood amino acid concentrations, micronutrient status. | 88<br>Mean age: 18.8 ± 11 years. | Total: -0.326. |
| **Choukair *et al.*, 2017[15], Germany** | Single centre, cross-sectional study. | Total BMD of distal and proximal radius Z-score, cortical and trabecular BMD Z-score, grip force, anthropometry, fractures, blood phenylalanine, PTH, 25-(OH-) vitamin $D_2$, serum calcium, serum phosphate, ALP, osteocalcin, TRP, urinary calcium/creatinine ratio, DPD crosslinks | 56<br>Age range: 11.8-41.5 years. | Distal radius total: -1.05.<br>Proximal radius total: -0.11.<br>Cortical: 0.12.<br>Trabecular: -0.18. |
| **Stroup *et al.*, 2017[28], USA** | Baseline results of cross-over trial. | BMD Z-score for total body, lumbar, femur, and radius, body composition, potential renal acid load (PRAL) from protein substitute, dietary intake, 24-hour urine samples. | 8<br>Age range: 16-35 years. | Mean Z-scores for the cohort not reported. |
| **Stroup *et al.*, 2018[17], USA** | Baseline results of randomised cross-over trial. | Total body, lumbar and femur BMD Z-score, body composition, PRAL from protein substitute. | 15<br>Age range: 15-50 years. | Males: total body: -0.9, lumbar: -1.3, femur: -0.7.<br>Females: total body: 0.2, lumbar: -0.4, femur: 0.4. |
| **Lubout *et al.*, 2019[19]. Netherlands** | Multicentre retrospective survey study. | BMD Z-score for lumbar, femoral neck, total proximal femur, radius and total body. Natural protein intake, calcium and vitamin D supplements, use of sapropterin dichloride, mean phe the year before the recent DXA scan, smoking and alcohol consumption. | 183 early treated PKU (ETPKU) adult patients. | Mean Z-score (+/- 1SD)<br>Lumbar: -0.527<br>Femoral neck: -0.324<br>Total proximal femur: -0.262<br>Radius: -0.298<br>Median Z-score<br>Total body: -0.400 |

## Appendix 1: summary of publications investigating bone health in PKU

| Fractures assessed | Physical activity assessed | Nutrient intake assessed | Author's Conclusions | Limitations |
|---|---|---|---|---|
| ✗ | ✗ | ✗ | Osteopenia and osteoporosis has been detected in the adult PKU population. The decrease in peak BMD in adult patients may be explained by long-standing dietary deficiencies or a primary defect in bone turnover inherent to the disease itself. | Misinterpretation of DXA Z-scores to diagnose osteoporosis and osteopenia.<br>Limited studies included which reviewed BMD. Limited information reported. Mean BMD z-scores not provided to allow comparison. |
| 20% fracture rate of 263 subjects. | ✗ | ✗ | Spine BMD is lower in PKU than control subjects, statistical significance not reported. Studies inconsistently controlled for reported smaller body size of PKU subject. Data lacking to show if lower spine BMD results in a higher fracture rate. The cause of low BMD in PKU is unknown. Suggestion of a clinical fracture rate of 20% among PKU subjects, fracture rates in controls are lacking. | Young cohorts. Inclusion of late diagnosed PKU patients and patients who liberalised their diet after the age of 8 years. These patients would likely have had reduced mobility associated with cognitive impairment and/or nutritional deficits which would increase their risk of lowered BMD. |
| ✗ | ✗ | ✗ | Mean BMD is within the normal range in PKU subjects, although mean BMD is lower in PKU patients compared to reference groups, statistical significance not reported.<br>90% of early treated patient with PKU are not at risk of low BMD.<br>Clinical significance of a slightly lower BMD Z-scores is unknown. | Adherence to dietary treatment has not been assessed in the systematic review.<br>Studies provided insufficient evidence to establish conclusions on BTM and other factors that may influence BMD including blood Phe concentrations, and nutrient intake.<br>Fractures were not included in the search terms when reviewing the papers. |
| ✗ | ✗ | ✓ | No evidence found for reduced BMD in children with PKU on specialised diets. Higher BMD was associated with calcium intake. In 19 participants, 3 had low BMD for chronological age (Z-score ≤ -2) measurement at either the hip or the spine and none had a low BMD at both the hip and the spine. | Mean BMD z-scores not provided to allow comparison.<br>Control group had other IMD and were not 'healthy' controls. |
| ✗ | ✗ | ✗ | No significant difference in BMD between groups ($p<0.05$). This could be because the cohort has maintained an adequate follow-up that includes sustained contributions of calcium and vitamin D provided by protein substitutes from diagnosis at NBS onwards. | Small cohort. Conference report therefore limited information provided. |
| ✗ | ✗ | ✗ | There was a trend for males to have slightly lower BMD but for the girls to have a higher total body fat. All children with PKU on a low phenylalanine diet were growing appropriately and had a normal bone density for age and size. | Mean total-BMD Z-score of whole study population not provided to allow comparison. Conference report therefore limited information provided. |
| 41.7% PKU participants had fracture(s), 38.2% in general population. | ✓ | ✓ | BMD Z-scores are within the normal range but lowered compared to the general population, statistical significance not reported. The clinical implications may be limited as none of the patients have osteoporosis as defined by ISCD. Lifetime fracture prevalence was normal. | The authors were unable to investigate association between dietary intake, blood concentrations of nutrients and BMD or fracture history. |
| ✗ | ✗ | ✓ | No subject had low BMD for chronological age (Z-score ≤ -2) which represents a lower prevalence of low BMD compared to previous reports.<br>Compliance with medical food (protein substitute) prescription was the strongest predictor of total BMD Z-score. | Fracture history and physical activity not assessed. |
| 63% of female PKU participants had fracture(s) compared to 71% in female study population. | ✗ | ✗ | The radial bone is characterised by inadequately reduced bone strength in relation to muscular force, reduced cortical thickness, and reduced total BMD at the metaphyseal site. These alterations indicate a mixed bone defect in PKU, both of which are due to primary alterations of bone metabolism and to secondary alterations in response to neuromuscular abnormalities. | Conclusions drawn on radial bone BMD only. Proximal radius, trabecular or cortical BMD were not significantly altered. 64% of study population took any protein substitute, no sub analysis of correlation of protein substitute intake and outcomes reported. |
| ✗ | ✗ | ✗ | 2 of 8 participants had low BMD-for-age (Z-score ≤ -2) and evidence of bone microarchitectural degradation. | Small cohort. Mean BMD Z-score of the cohort for any BMD measurement not provided to allow comparison. No participant had low BMD at both femur and lumbar. |
| ✗ | ✗ | ✗ | Males with PKU have lower total body and femur BMD compared with females with PKU which may be related to higher intake of AA-based protein substitutes and greater calcium excretion. | Small cohort. No significant differences found between male and female PRAL, g PE AA-based protein substitute, RNAE, magnesium or sulphate to support hypothesis that higher intake of AA-based based protein substitutes result in lower BMD. |
| Fractures described in 30 patients (16.4%), which is significantly lower than the estimated age-standardized fracture prevalence of 38.2% for England. | ✗ | ✓ | Most ETPKU patients have a BMD within normal range, with only a maximum of 5.5% having a low BMD. A DXA scan should potentially be requested in PKU patients aged >35-40 years and in those PKU patients considered to be at increased risk for fractures. | Most participating centres routinely performed a DXA scan in all patients, some centres only carried out a DXA scan in a selection, this may cause bias in under or over estimation of low BMD. Additional analysis on risk factors unable to be carried out due to low prevalence of low BMD. Described fracture prevalence may be underreported as based on chart studies which are less reliable. |

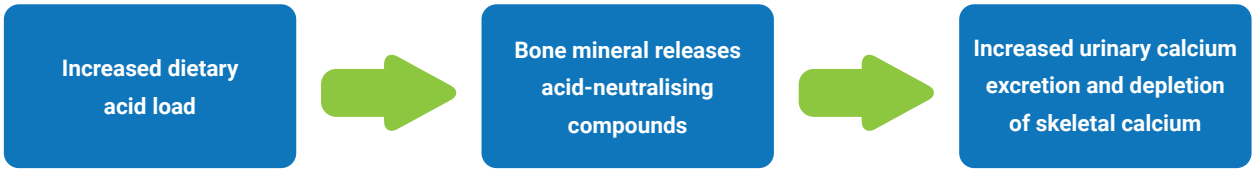
## 9 Appendices

| Author and year | Protein | Vitamin D (25(OH)D) | Calcium, phosphate and magnesium |
|---|---|---|---|
| **Demirdas *et al.*, 2015**[11] | Total protein intake did not correlate with BMD. Correlation with protein substitute intake was inconsistent, one study reported a positive correlation and one study reported no correlation. | Vitamin D status did not correlate to BMD. Vitamin D intake was not assessed. | Lower plasma calcium concentrations reported in children with PKU but impact on bone ambiguous. Blood phosphorus and magnesium concentrations not linked to bone status. |
| **Geiger *et al.*, 2016**[13] | Significant positive correlation between protein substitute intake and spine BMD. | No significant difference in 25(OH)D levels between IEM patients and the control children. No correlation between serum 25(OH)D and BMD. | Significant, positive correlation between calcium intake and spine BMD. |
| **Demirdas *et al.*, 2017**[16] | All patients had protein intakes above minimum safe recommendations. | Vitamin D intake was below minimum requirements in 20% of participants. Vitamin D status was low in 14% of participants. | Not reported. |
| **Coakley *et al.*, 2016**[18] | Positive correlation found between compliance with protein substitute and actual protein substitute intake (gPE) and BMD. Total BMD Z-score was significantly negatively correlated with protein substitute prescription. Dietary phenylalanine and total protein intake were not correlated to BMD. | Normal vitamin D status found in 84% of their participants. Serum vitamin D status was not correlated with BMD. BMD (adjusted for BMI) was positively correlated to vitamin D intake. | BMD (adjusted for BMI) was positively correlated to calcium intake. |
| **Choukair *et al.*, 2017**[15] | Did not report correlation between BMD and protein substitute intake. 64% of participants took any protein substitute. | Vitamin D deficiency or insufficiency reported in 83% of the cohort. No correlation between serum vitamin D concentration and BMD. | Not reported. |
| **Stroup *et al.*, 2017**[28] | Intake of PE from AA-based protein substitute was negatively correlated with total body BMD (p=0.04) but not lumbar BMD. | Serum concentrations of 25(OH)D were within normal limits. | Serum concentrations of calcium were within normal limits. |
| **Stroup *et al.*, 2018**[17] | Mean total femur DXA Z-score was negatively correlated with intakes of AA-based protein substitutes (p=0.048) but not spine or total body. | Not reported. | Not reported. |
| **Lubout *et al.*, 2019**[19]. | It was not possible to draw conclusions on the exact amount of natural protein intake and medical food protein intake. It was not clear what type of medical foods were being consumed. | Low vitamin D concentration reported in 32% of 173 patients. Vitamin D supplementation documented in 26% of 162 patients. An association with BMD was not found for either. | Calcium supplementation reported in 19% of 161 patients but no association with BMD found. |

* See **appendix 3** "What is potential renal acid load (PRAL) and how does it relate to PKU?"

### Appendix 3 What is potential renal acid load (PRAL) and how does it relate to PKU?

PRAL is a measure of the acid-base load of foods and estimates renal net acid excretion (RNAE)[47]. It is suggested that increased acid load has a negative impact on bone health by the following process[28]:




PRAL is a controversial theory and is not widely accepted[46, 47]. It is reported that a causal association between dietary acid load and osteoporotic bone disease is not supported by evidence[46, 47].

## Appendix 2: summary of publications investigating dietary factors in patients taking AA-based protein substitutes

| Other findings | Author's Conclusions | Limitations |
|---|---|---|
| n/a | Dietary compliance and dietary intake assessed as reported protein substitute intake, total protein or phenylalanine intake were not correlated to BMD or BTM. Vitamin D status does not seem to influence BMD. | The impact of overall protein status, including biological value of intact versus protein substitute and percent of total protein derived from protein substitute on bone were not considered by any study included in the review. Micronutrient intake and correlation to BMD was not investigated. |
| n/a | BMD was a significantly correlated with dietary calcium and protein substitute intake, suggesting that consumption of protein substitute, which provide most key nutrients important for bone health, play a crucial role in developing peak bone mass among patients with PKU. No evidence of low serum vitamin D in their population of children with IEMs compared to control children. | Small cohort of 12 PKU patients completed 3-day food diaries which lowered statistical power to investigate relationships between dietary intakes and BMD. |
| 58% of participant's total fat intake was below minimal recommended 20% of energy. | Dutch patients with PKU on long-term dietary treatment have a near normal nutrient status, however, supplementation of micronutrients of which deficiency may be deleterious (e.g. vitamin D and selenium) should be considered. | The authors were unable to investigate association between dietary intake, blood concentrations of nutrients and BMD or fracture history. |
| BMD was significantly negatively correlated with dietary carbohydrate intake, dietary sugar intake, total glycaemic load and caffeine intake. BMD was negatively correlated with DEXA scans being taking in winter months. | BMD Z-score was most positively associated with compliance with protein substitute prescription and dietary vitamin D intake and most strongly negatively correlated with caffeine intake and total glycaemic load. Promoting optimal protein substitute compliance may be a feasible strategy to improve BMD Z-score. | 3-day food diaries do not represent long-term food intake patterns. |
| Not reported. | No BMD parameter was related to serum 25(OH)D concentrations. Hence, to what extent the high prevalence (83%) of vitamin D deficiency or insufficiency in this PKU cohort contributes to the altered macroscopic bone architecture cannot be assessed. | Nutritional intakes and association with markers of bone health were not assessed. Large proportion of the cohort not taking any protein substitute which was not included in sub analysis of results. |
| AA-based protein substitutes provided 1.5-2.5-fold higher potential renal acid load (PRAL)* compared to GMP-based protein substitutes. | The authors established that AA-based protein substitutes provided a high PRAL and the high PRAL* was associated with higher urinary excretion of RNAE, calcium, magnesium and sulphate, which was considered likely to contribute to skeletal fragility in PKU. | Small cohort. High-sodium, low- PRAL* AA-based protein substitutes not included. Correlation between PRAL* and mean total BMD for total cohort not reported. |
| PRAL determined for protein substitutes. Correlation between PRAL* and BMD not reported. | Males with PKU have lower total body BMD Z-scores and may be at greater risk for osteoporosis than females with PKU. The authors hypothesised that low-normal BMD Z-scores found in male participants may be related to low-normal lean mass and/or higher intakes of AA-based protein substitutes with a correspondingly greater loss of urinary calcium. | Small cohort. Correlation between PRAL* and mean total BMD for total cohort not reported. No significant difference between male and female PRAL intake to support hypothesis that decreased BMD in males related to increased intake of high PRAL protein substitute. |
| Of 106 patients, 22% smoked and 26% consumed on average > 2 units alcohol per day. No association found with BMD. | No statistically significant differences were found in possible risk factors between patients with low BMD and patients with a BMD within normal range. | Dietary factors were derived from patient charts, therefore micronutrient intakes not assessed and not complete enough to draw conclusions on total protein and medical food protein intakes. |

Stroup *et al* in 2017 and 2018[17, 28] applied the PRAL theory to the PKU diet and implicated the PRAL value of AA-based protein substitutes as a cause of poor bone health in PKU. The calculation* that was used to determine PRAL of protein substitutes in these publications is heavily influenced by the minerals and electrolyte content of the products, the calculation only includes 2 amino acids. A high PRAL calculation for the AA-based protein substitutes examined in these studies were significantly influenced (p=0.006) by the higher sodium content of the GMP-based compared to amino acid-based protein substitutes.

The correlation between BMD and PRAL value of the protein substitutes, which would support the hypothesis that high PRAL reduces BMD, was not reported in either publication. It was reported that the correlation intake of high PRAL protein substitutes and BMD measures did not reach statistical significance[17, 28].

*The calculation[28] reference. PRAL = (2 × (0.00503 × mg Met/d)) + (2 × (0.0062 ×mg Cys/d)) + (0.037 × mg phosphorus/d) + (0.0268 ×mg chloride/d) − (0.021 × mg potassium/d) − (0.026 ×mg magnesium/d) − (0.013 × mg calcium/d) − (0.0413 ×mg sodium/d).

## Notes

## Notes

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A Nestlé Health Science Company

Vitaflo International Ltd,
Suite1.11, South Harrington Building,
182 Sefton Street, Brunswick Business Park,
Liverpool L3 4BQ, UK

**+44 (0)151 709 9020**
**www.vitafloweb.com**



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