MU METABOLIC UNIVERSITY

MU Webinar Series:

PKU and Bone Health—A Review

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Supported in part by:
NUTRICIA Metabolic Nutrition

Objectives

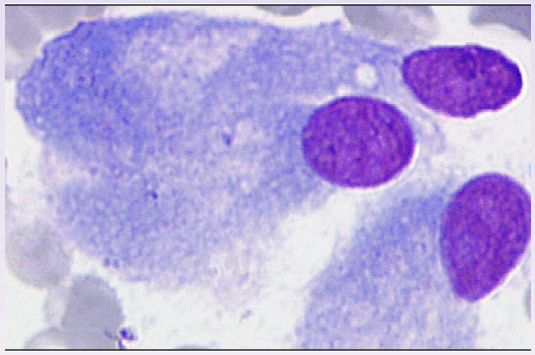
- Provide primer on bone metabolism
- Review possible hypotheses for bone issues in PKU
- Review selected publications on bone and PKU
- Review new research and findings

Function of the Skeletal System

- Support
- Protection
- Movement
- Mineral storage
- Blood cell formation (hemopoiesis)
- Triglyceride storage

Bone cells that aid in remodeling

Osteoblast



Builds new bone

Osteocyte

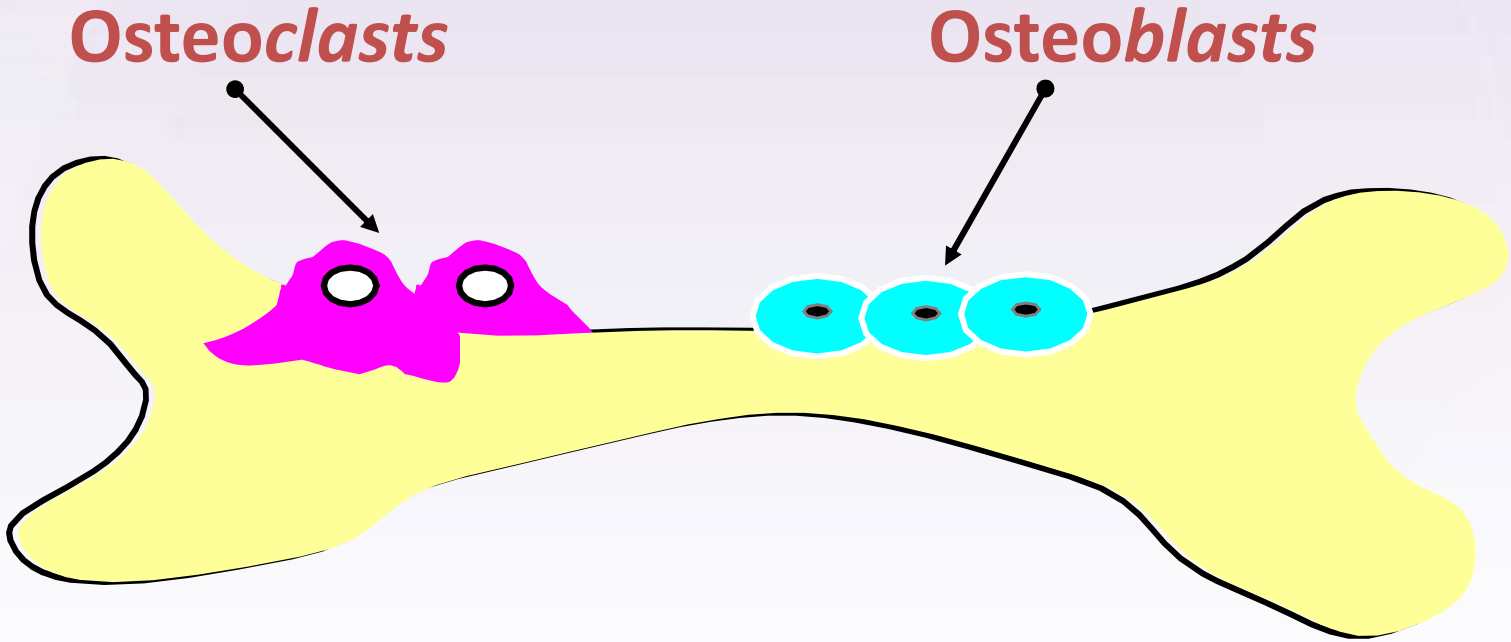
Mature bone cell

Osteoclast

Eats bone

Remodeling

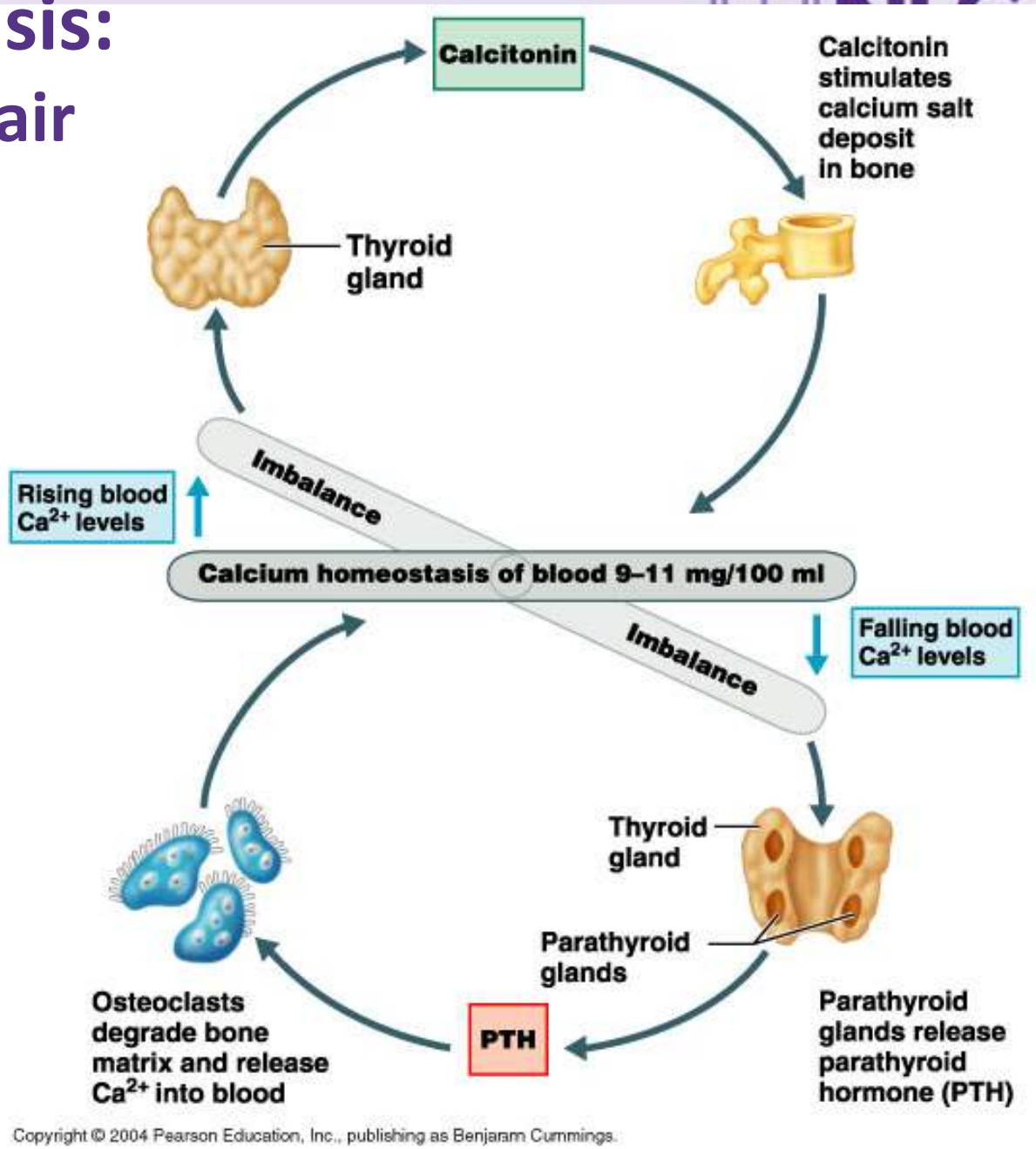
Osteoclasts **erode the bone surface, dissolving mineral and matrix.**



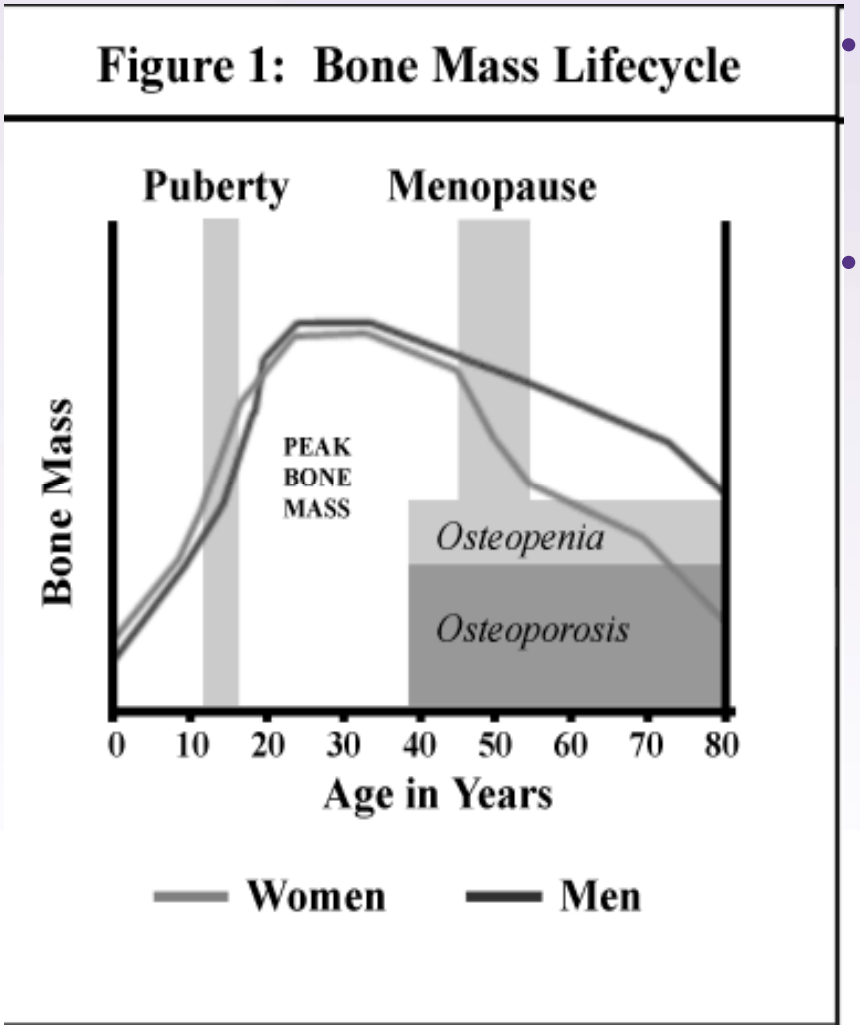
Osteoblasts **build new bone, laying down collagen and minerals.**

Bone Homeostasis:

Remodel and Repair

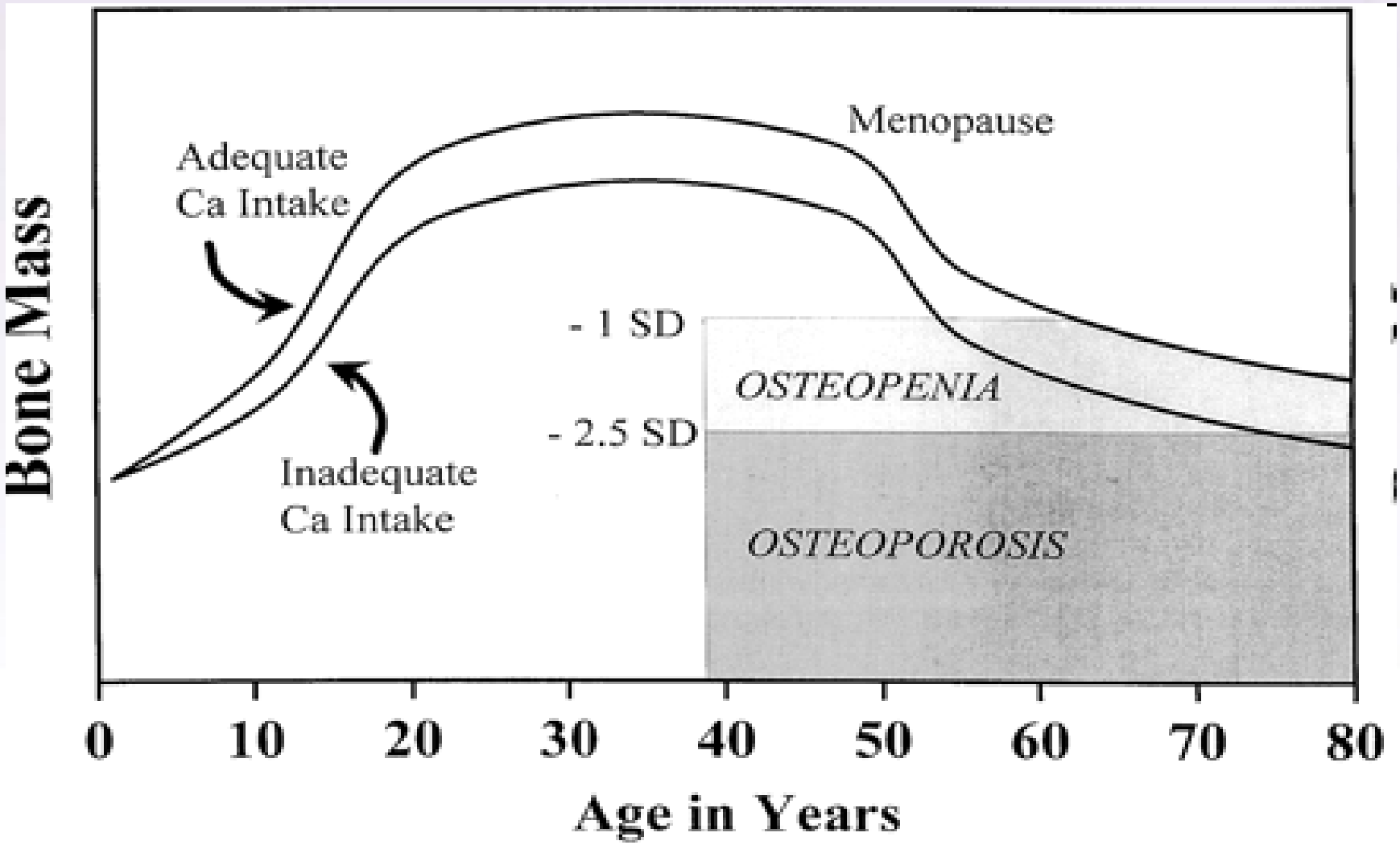


Normal Bone Acquisition



- 95% of total bone mineral density by age 18 years
- Factors that Affect Bone Mineral Density
  - Nutrition
    - Ca/Vit D/protein/Vit K/Vit A
  - Exercise (weight bearing)
  - Sunlight
  - Hormone fxn
  - Health status (well vs infection)
  - Genetics
  - Lifestyle
    - Smoking, alcohol

Relationship of Calcium Intake to Bone Mass



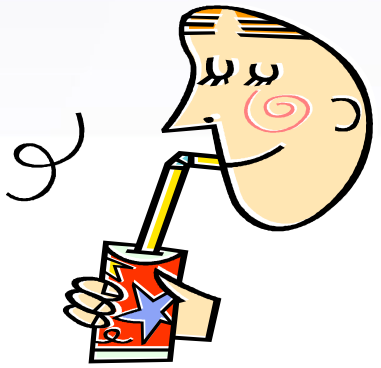
Lets Look at PKU! What we know

- #1: Phenylketonuria is a lifelong chronic autosomal recessive disorder
- #2: For most patients lifelong diet substitution of HBV protein foods with free amino acids devoid of phenylalanine
- #3: Majority (85%) dietary protein comes synthetic sources
- Majority of macro-and micronutrients, including bone-related nutrients come from non-food sources
- Patients with PKU do not have access to a variety of foods
- Compliance to a life-long diet with medical foods is poor with age.
  - Medical foods are not replaced with nutrient-rich foods
  - Nutrient deficiency has been reported in patients not adhering to diet

Reported Causes of Bone issues in PKU

- Protein/Energy Malnutrition (early years)
  - More research showing importance of protein
- Poor Dietary Adherence
  - Chronic hyperphenylalaninemia
  - Decreased macrominerals
  - Decreased medical food/formula intake
    - Al Qadreh: ↑ med food => ↑ BMD

    Perez-Duenas 2002 (calcitriol 0.25 ug/d increased BMD)
- Inherent in PKU? (deGroot et al 2012)
- Protein Source (Solverson et al 2012)



Published studies on BMD and PKU

- Carson, (1990) *Pediatr Radiology*
- M. Mc Murry (1992) *Am J Clin Nutr.*
- J. Allen (1994) *Am J Clin Nutr*
- L. Hillman (1996) *Eur J Pediatr*
- Schwahn (1998) *Am J Clin Nutr*
- *A.* Al-Qadreh (1998) *Acta Paediatr*
- J. Zeman (1999) *Acta Paediatr*
- H. Przyrembel (2000) *Eur J Pediatr*
- P. Barat (2002) Eur *J Pediatr*
- S. Yannicelli (2002) *J Inherit Metab Dis.*
- J. Ambroszkiewicz *(2004) Eur J Pediatr; 2004*
- D. Modan-Moses (2007) *J Inherit Metab Dis*
- Roata I et al 2010
- De Groot MJ et al JIMD 2010
- Nagasaka H et al 2011
- Demirdas S et al, Orphanet J Rare Dis, 2015

PKU and Bone: Not a New Concern

• **1962** Feinberg and Fisch (Radiology)	• Diet not a “proved” direct cause • Patients poorly managed had poor radiographs (Compliance)
• **1964** Murdoch and Holman (Am J Dis Child)	• Direct correlation bw bone-diet- se alk phosphatase • Se alk phos nL after adeq pro intake
• **1967** Holt and Allen (Annales de Radiologic)	• ½ patients had some degree of osteoporosis; 1/3 had delayed skeletal maturity (32 children 2-10) • Defect related to diet and not disease itself (Diet)

Published Studies on PKU and Bone

- 26 PKU patients (1.9 - 25.5 years); 164 controls (3 - 16 years) were studied by single photon absorptiometry

- Patients with PKU and controls had similar increase in bone mineral content (BMC) up to 8 years of age.
  - After 8 years of age BMC in patients with PKU started to fall below that of controls.
    - Below 8 years- BMC similar to controls
    - Above 8 years- lower BMC compared to control

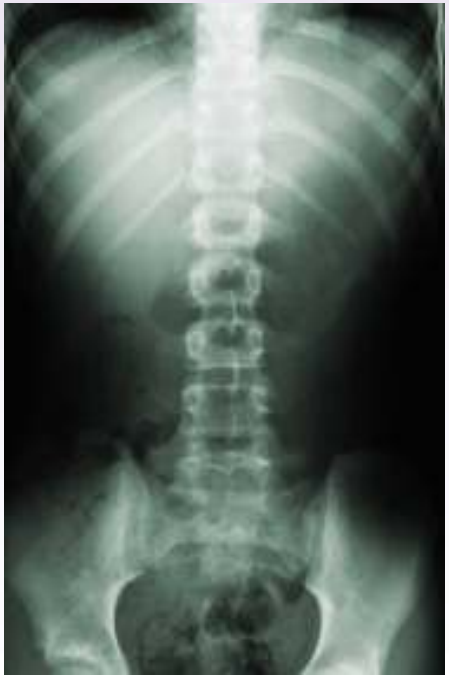
- Plasma CA/P were not significantly different, but ALK phosphatase was significantly lower in patients with PKU than in controls

First indication that high plasma PHE levels negatively affect BMC

Source: McMurry et al: Am J Clin Nutr (1992).

Published Studies on PKU and Bone

- 32 prepubertal children with PKU (females < 10 years, males < 12 years) and 95 controls were studied by dual energy X-ray absorption (DEXA)
- No significant difference in dietary intake Ca/P/Mg b/w groups
- Total BMD and spinal BMD were significantly decreased in children with PKU
- No correlation with serum PHE and BMD

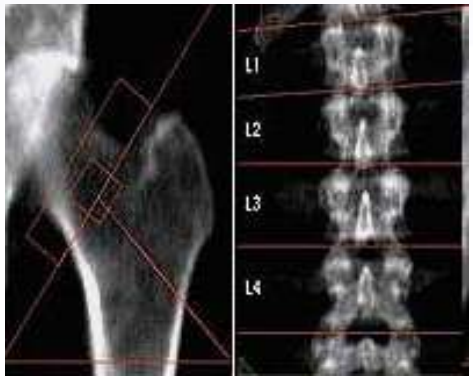


Source: Allen et al: Am J Clin Nutr (1994).

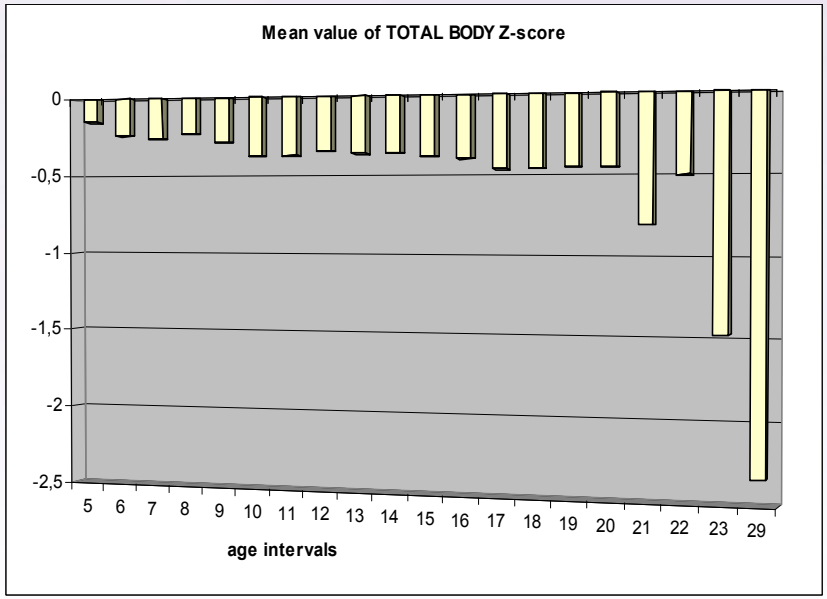
Published Studies on PKU and Bone

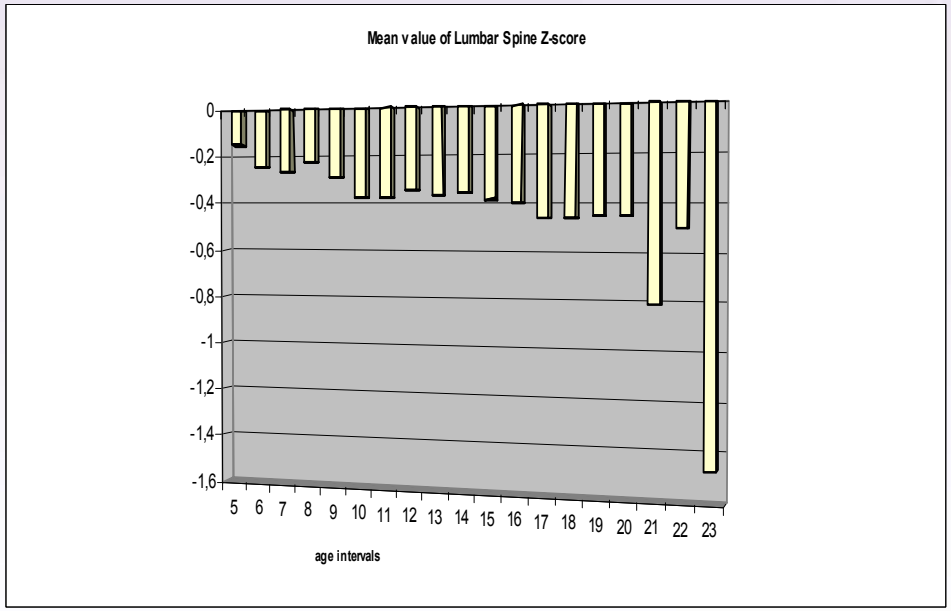
- 11 patients with PKU (age: approx. 11 ± 4.2 years)
- Analysis: Retrospective look at DEXA scans
- Results (PKU patients compared to age matched controls)
  - Decreased BMD (lumbar spine; lower extremities) compared to age-matched controls
  - Decreased serum Ca/Mg
  - Bone formation markers: decreased bone alkaline phosphatase and osteocalcin
    - (Similar results found by Ambroszkiewicz 2004 (Eur J Ped. 163:177-178)
  - Bone resorption markers (urine Ca/creatinine)= no difference
- No relationship in serum PHE with protein and mineral intake and bone measures

Source: Hillman et al, Eur J Pediatr (1996)



Mean Z-score BMD Decreased With Age And Was Significantly Lower Among Young Adults





A. Lange. Polish PKU Working Group, ICIEM, Aug. 2009

Acosta, Yannicelli, Pazquali 2002

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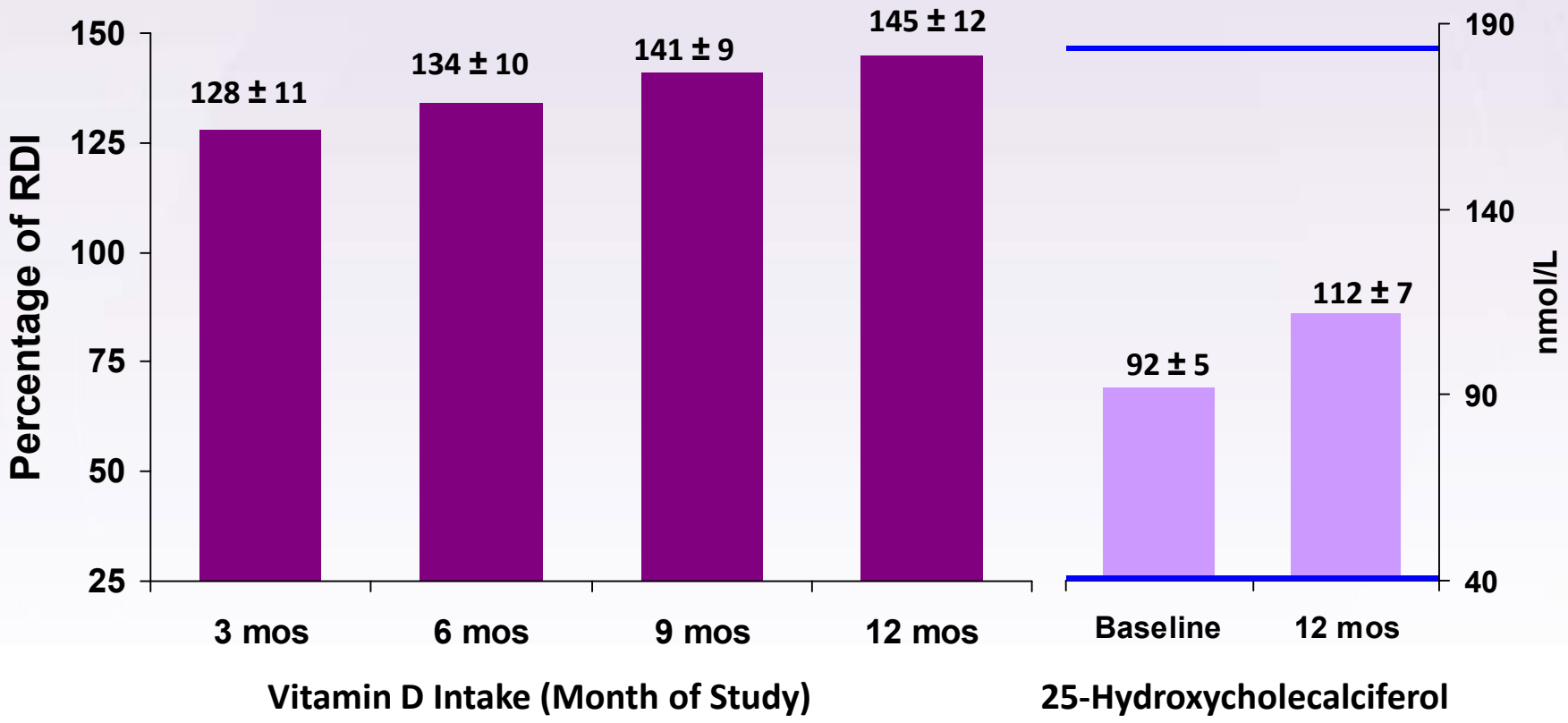
- **Objectives:**
  - Define a non-invasive biochemical marker of bone turnover useful to monitor bone metabolism in patients with PKU.
  - Correlate this parameter with nutrient intake, diet, and other markers of bone metabolism.
- 48 patients with PKU, age 2 <13 years, participated in the study.
  - First morning urine samples were collected at three month intervals for pyridinium cross-links analysis.
  - CA/P, ALK phosphatase, plasma amino acids were measured at each visit.
  - Protein and mineral intakes were determined by diet calculation.

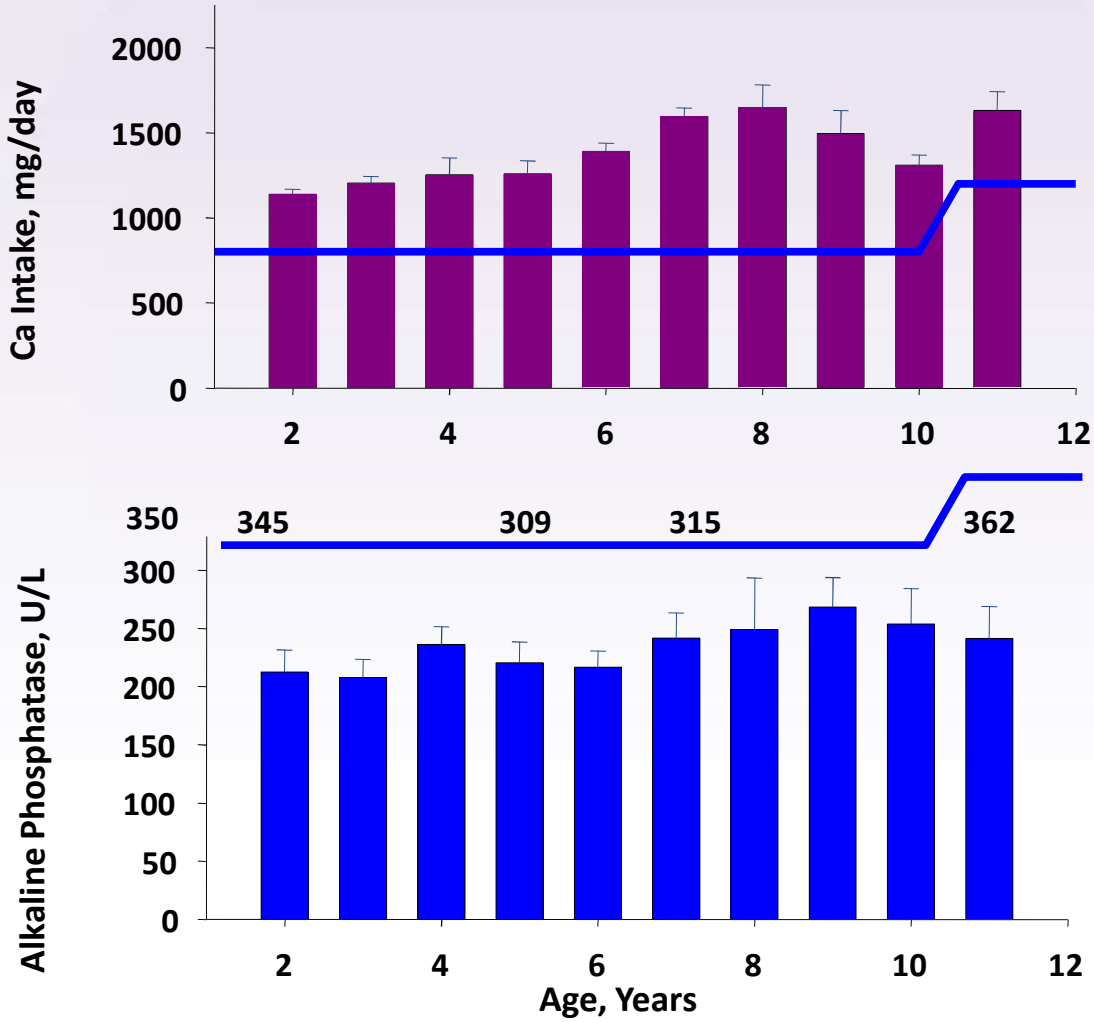
Vitamin D Intakes and Serum 25-Hydroxycholecalciferol Concentrations



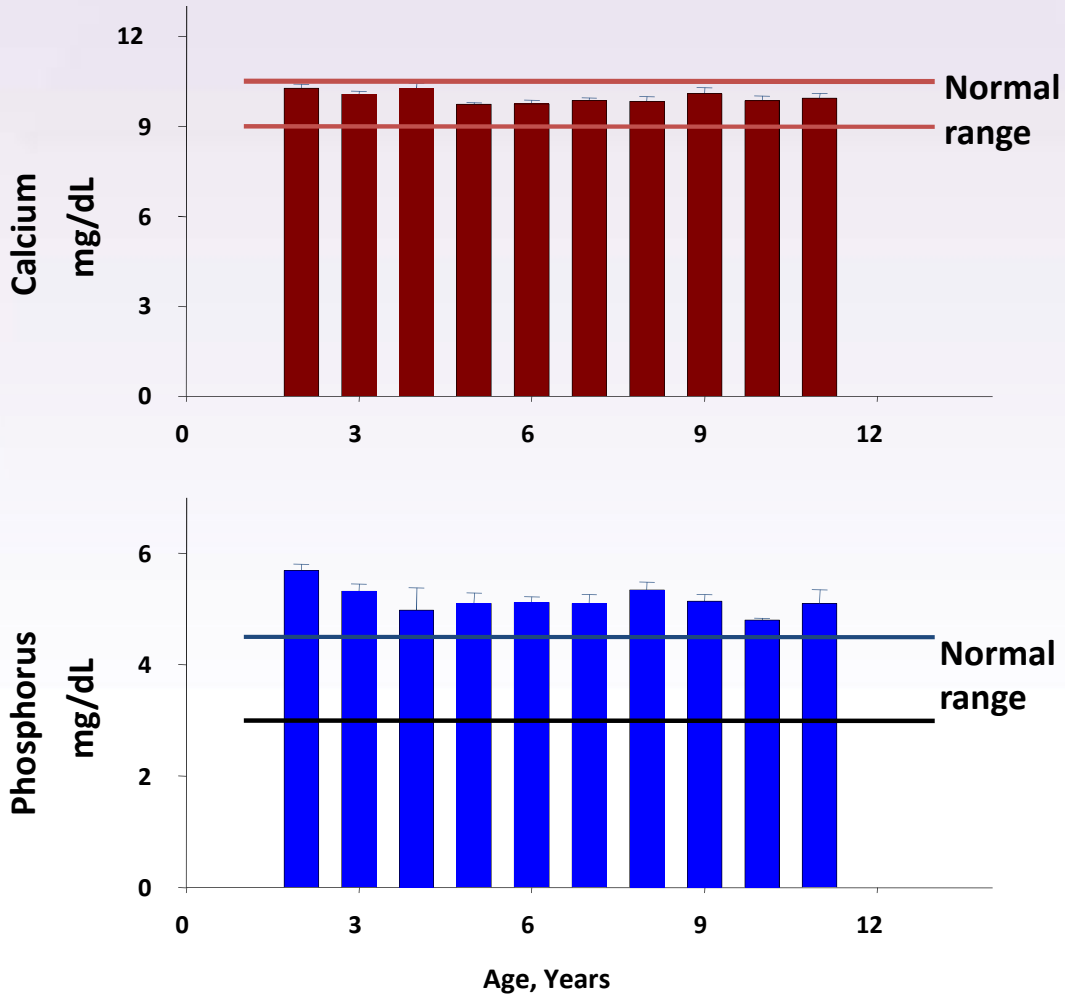
Values are mean ± SEM.

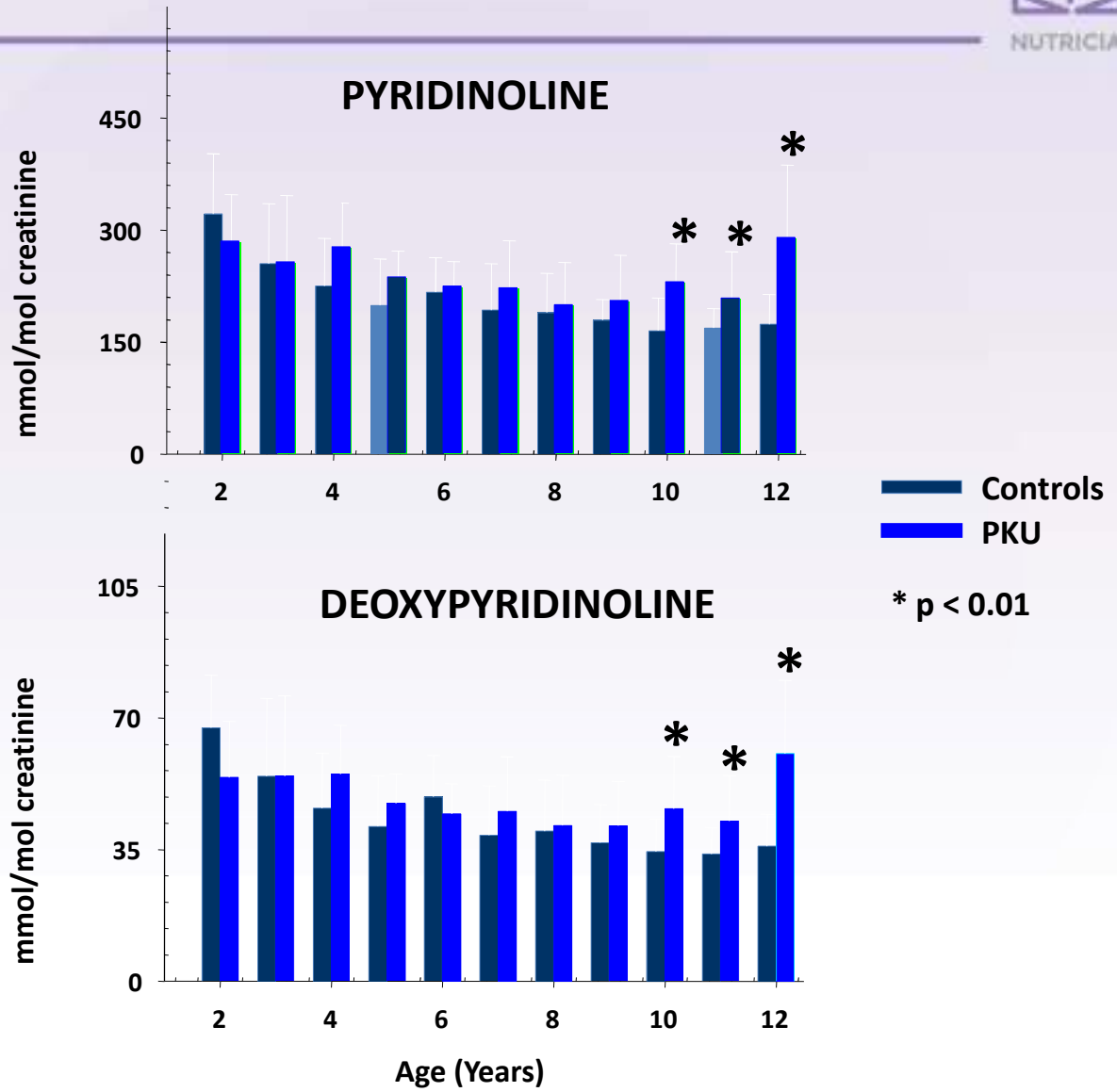
Reference Range

Changes With Age in Calcium Intake and Alkaline Phosphatase in Patients With PKU

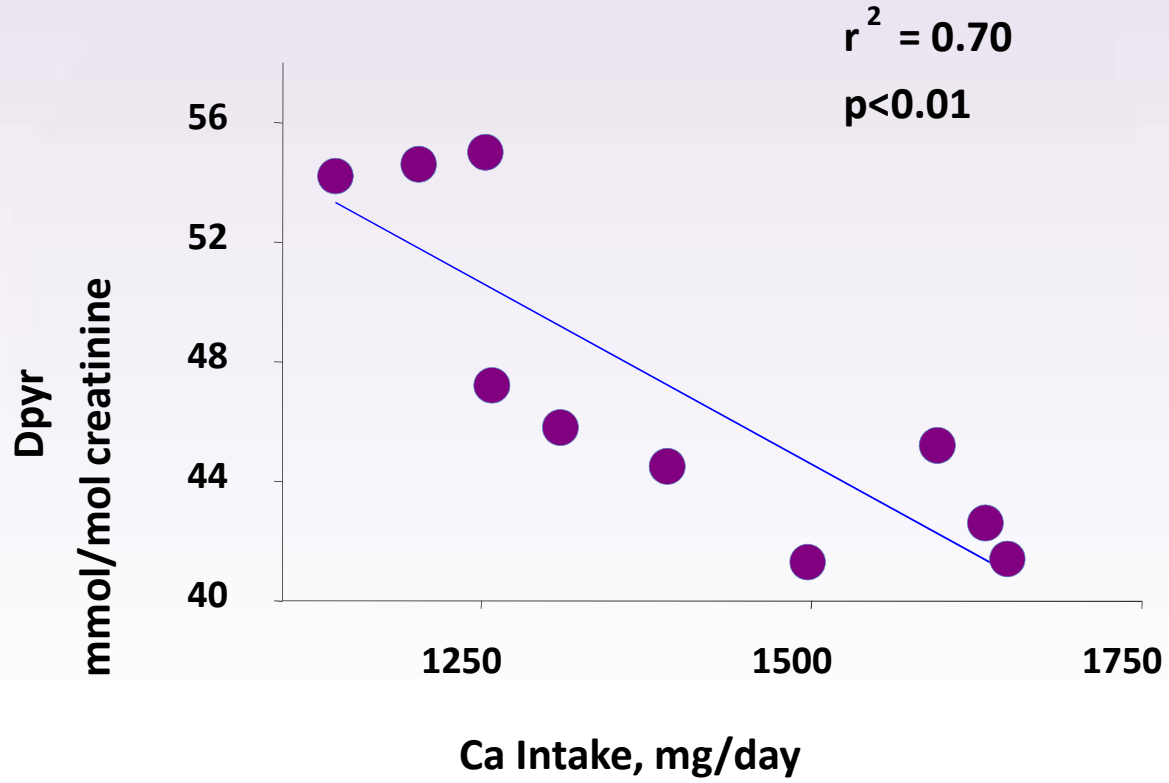


Plasma Ca and Phos Concs in PKU




PYRIDINOLINE
mmol/mol creatinine
450
300
150
0
2
4
6
8
10
12
*
*
*
Controls
PKU
* p < 0.01
DEOXYPYRIDINOLINE
mmol/mol creatinine
105
70
35
0
2
4
6
8
10
12
*
*
*
Age (Years)

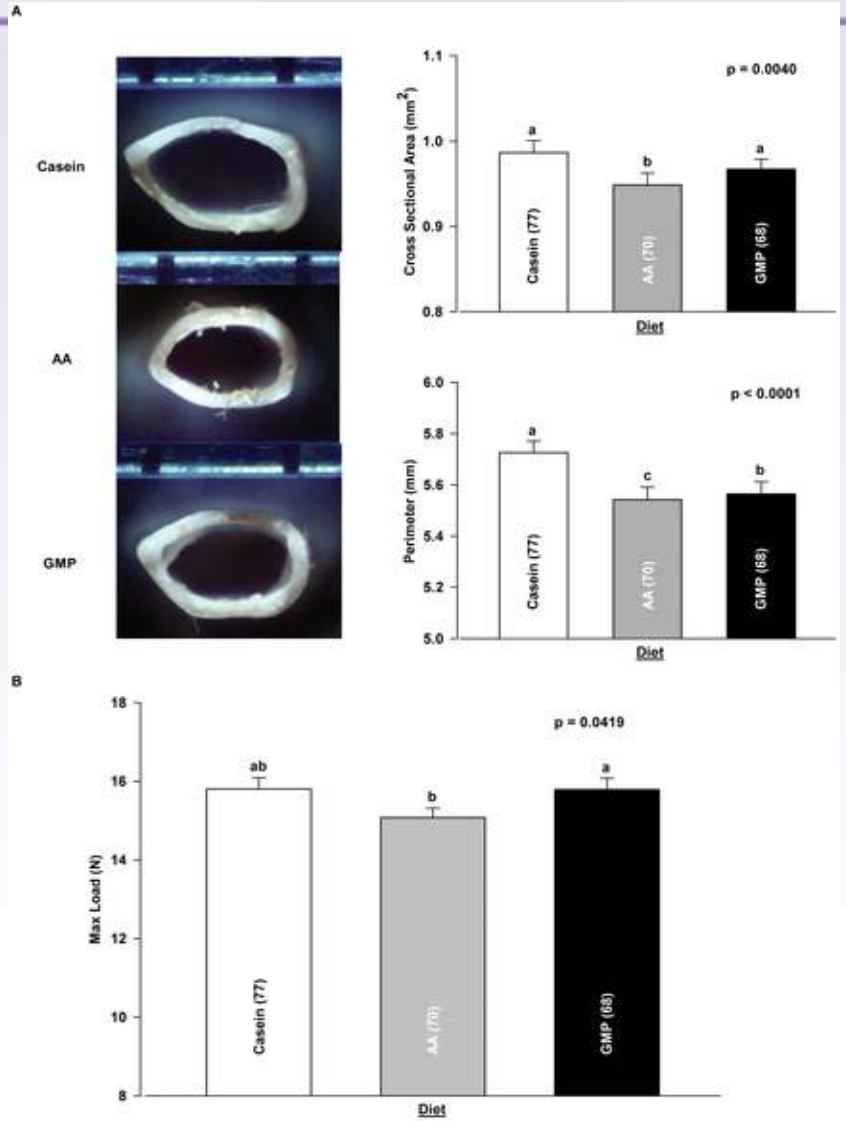
Correlation Of Urinary Deoxypyridinoline With Calcium Intake



Conclusions

- Bone resorption is significantly higher in PKU pts than controls after 8 years of age, suggesting increased bone turnover.
  - deGroot et al 2012 reported higher bone turnover in PKU pts
- Increased dietary CA intake was correlated with decreased bone resorption. Higher Ca = Less resorption
- Compliance with diet decreases before changes in urinary pyridinium cross-links, suggesting a causal relationship.

Diet Modifies Femoral Size And Strength In WT And PKU Mice.



Solverson P, Murali SG, Litscher SJ, Blank RD, et al. (2012) Low Bone Strength Is a Manifestation of Phenylketonuria in Mice and Is Attenuated by a Glycomacropeptide Diet. PLoS ONE 7(9): e45165. doi:10.1371/journal.pone.0045165
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0045165

Conclusion and Limitations

- WT mice differ from PKU mice –genotype
  - Yield load, pyridinium crosslinks
  - PKU femora is more fragile then WT
- GMP attenuated the PKU bone phenotype

- Limitations:
  - Bending test area is not where clinical fractures occur
  - Only measured cortical bone, not active bone
  - Mice were started after weaning
  - No data on whether isonitrogenous or coprophagia controlled
- It's a Mouse Study!

Protein Intake and Bone:PKU

- Cross-sectional observational study
- 43 pts with PKU
  - 41.9% Male
  - Mean age 17.6 yrs (7-41 years);65% <20 yrs of age
  - 14 pts dx'd late
  - 12 pts on BH4 for avg. 7.1 years (1 classic, 6 mild and 5 moderate
- Measurements: Nutrition assessment, bone densitometry, molecular and biochemical studies, physical activity

Miras et al MGM 2013;108:149-154

Protein Intake and Bone: PKU

Results:

- 14% of pts had mineral bone disease
- Amount of Dietary Protein: Pts with osteopenia and/or osteoporosis (n6) had avg total protein intake less than pts without 14.33g/day +/8.5 vs 21.5 +/-20.85
- Type of Protein: Pts on saproterin consumed more natural protein and had no identified MBD. Also, consumed more dietary PHE.
  - Caveat: observational and not causal relationship
- Pts on saproterin had higher bld levels of DHA and EPA
  - Question on role of oxidative stress and bone

Table 2
Nutritional characteristics of MBD patients.

Patient number (age)	kcal/day	Total proteins (g/kg day)	Natural proteins (g/kg day)	Ca intake mg/day (N: 800 mg/day)	P Intake mg/day (N :800 mg/day)	Phe(b) medium (range)
13 (30 years)	2927 (2000–3300)	1.5 (0.84)	0.19	1489	1537	475(394–537) (<600 μmol/L)
16 (26 years)	1506 (1400–2500)	1.4 (0.84)	0.31	1271	1405	688(428–900) * (<600 μmol/L)
30 (8 years)	1408 (1300–2300)	2.8 (0.87)	0.73	1179	1227	148.0 (72–405) (<360 μmol/L)
33 (41 years)	2479 (2000–3300)	0.52 (0.84)	0.46	1008	1179	762(602–1018)* (<600 μmol/L)
36 (10 years)	2000 (1650–3300)	1.8 (0.92)	0.26	984	1104	518 (430–605) (<600 μmol/L)
42 (31 years)	1363 (2000–3300)	0.38 (0.84)	0.19	414*	493*	459.0 (<600 μmol/L)

In parentheses: recommended daily allowances for age. Total proteins/kg: values in parentheses are the WHO safe level recommendations for the typical population[17].
N: normal; b: blood; *: values outside of recommended range.

Miras et al MGM 2013;108:149-154

Vitamin D & PKU

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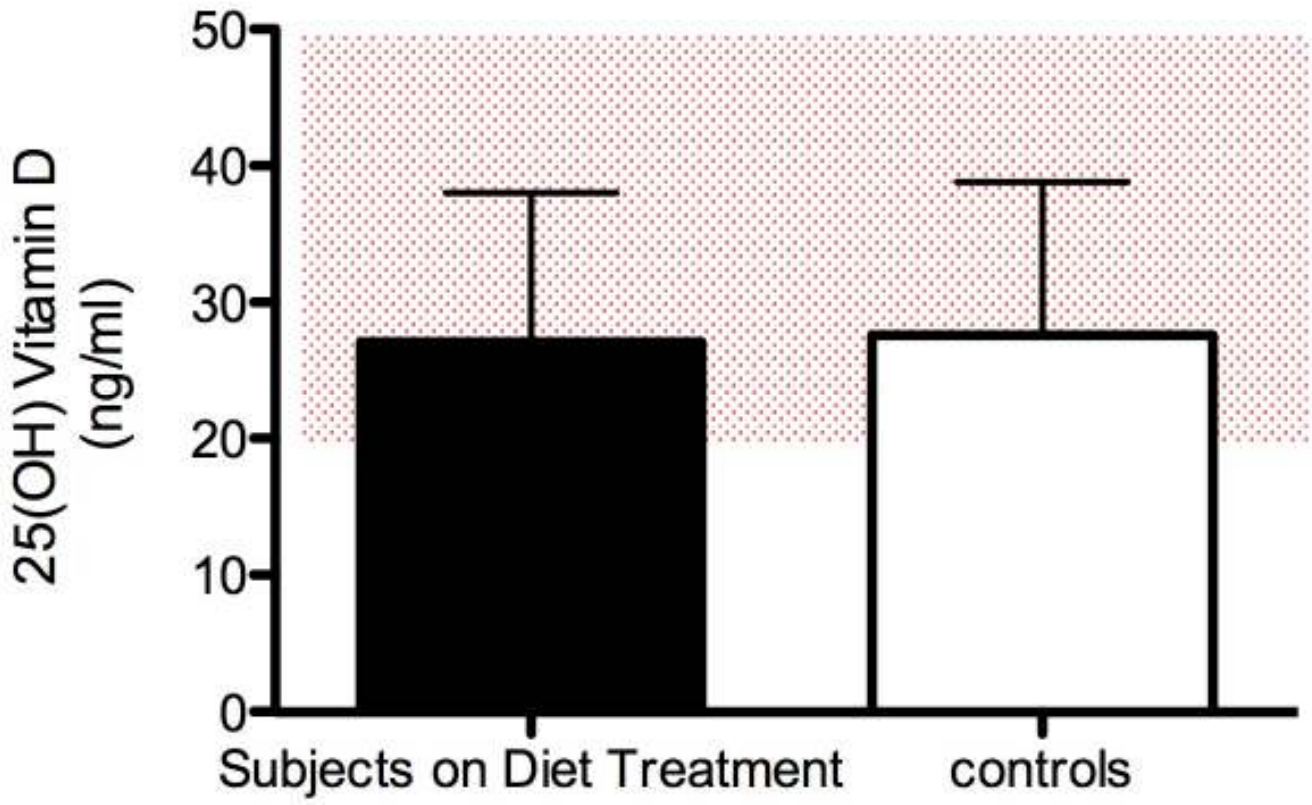
Baseline Characteristics and comparisons between cases and controls

Characteristic	Case (n=92)	Control (n=445)	p-value
Mean (±SD) age on draw date in years	12.5 ± 3.47	14.6 ± 3.39	<0.001
Sex, n (%)			0.264
Male	47 (51.1)	199 (44.7)	
Female	45 (48.9)	246 (55.3)	
Region^, n (%)			0.531
Northwest	60 (65.2)	318 (71.5)	
Southwest	24 (26.1)	101 (22.7)	
Northeast	4 (4.3)	10 (2.2)	
Southeast	4 (4.3)	16 (3.6)	
Season of blood draw, n (%)			0.331
Winter (10/1--5/31)	65 (70.6)	291 (65.4)	
Summer (6/1--9/30)	27 (29.4)	154 (34.6)	
Mean (±SD) [25(OH)D] in serum in ng/ml	27.1 ± 10.9	27.6 ± 11.2	0.672

^ N/S demarcation made relative to 45th parallel; E/W demarcation was the Cascade mountain range (determined via GIS).

Courtesy of Melanie Gillingham

Vitamin D

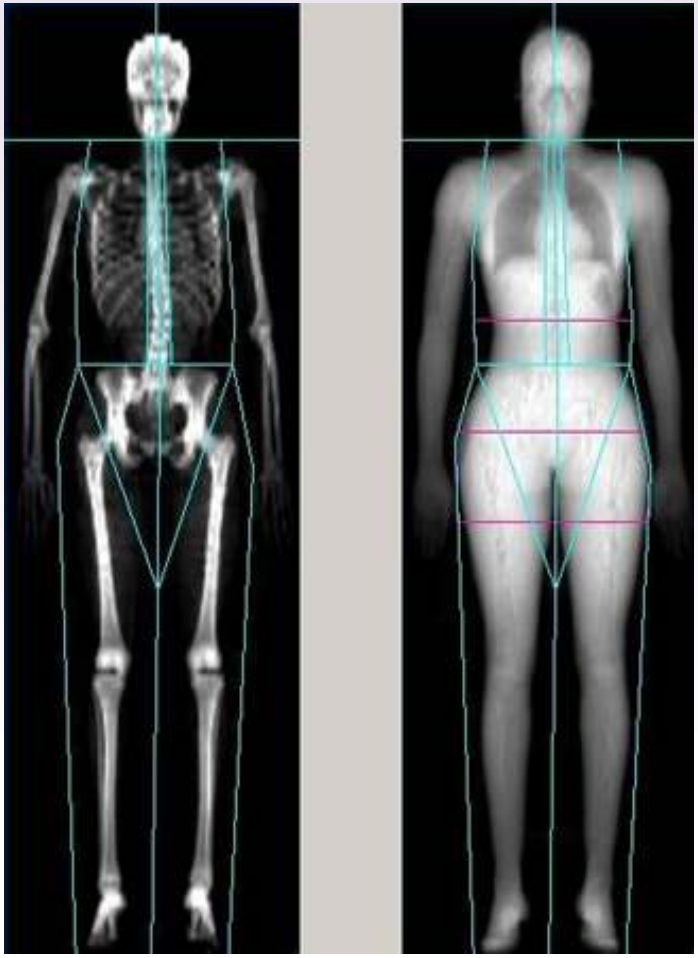


Normal vitamin D in both patients on diet treatment and controls.

Courtesy of Melanie Gillingham

PKU & Bone Mineral Density

- Measured bone density of 20 subjects with PKU
- Evidence of osteopenia among ¼ of subjects
- All had normal OH-vitamin D concentration & normal PTH



Courtesy of Melanie Gillingham

Conclusion: Vitamin D

- Deficiency rare on diet treatment
- Some evidence for low bone mineral content
- Unknown etiology
- Continue to promote
  - Good metabolic control
  - Intake of medical foods
  - Moderate weight bearing exercise

Gut Microbiota

Gut **microbiota** is an assortment of microorganisms inhabiting the length and width of the gastrointestinal tract

Gut **microbiota (formerly "gut flora")**:

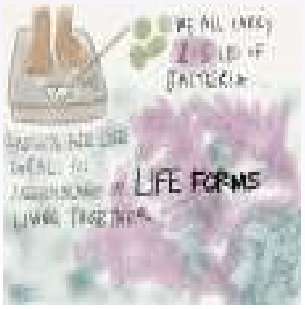
~100 *trillion* living bacteria

More bacterial cells than "self" cells!

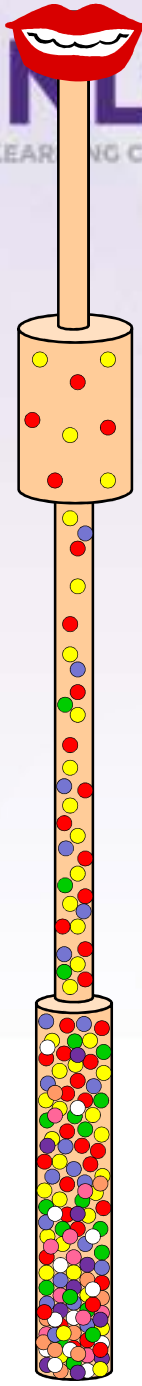
- Mostly ***commensal*** species: symbiotic, "friendly"
- *At least 1000 different species; more than 3 million genes (150 x more than human genes)*
- Changing constantly
- 2/3 specific to each one of us → your identity card more than your genes!
- Populations differ along the GI tract

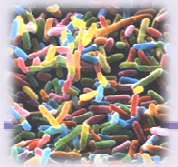
  More species/variety further along

  More overall numbers further along



Microbiota can weigh up to 2 kg

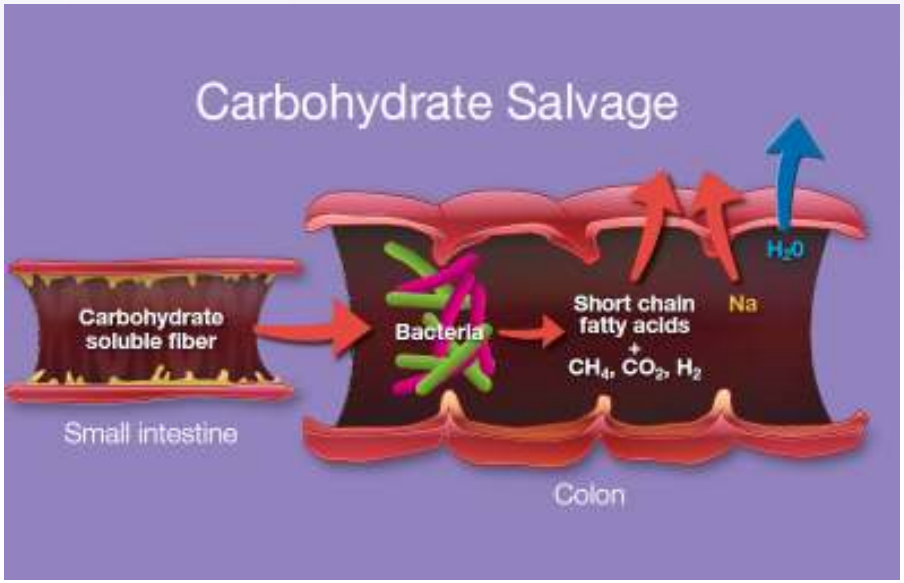


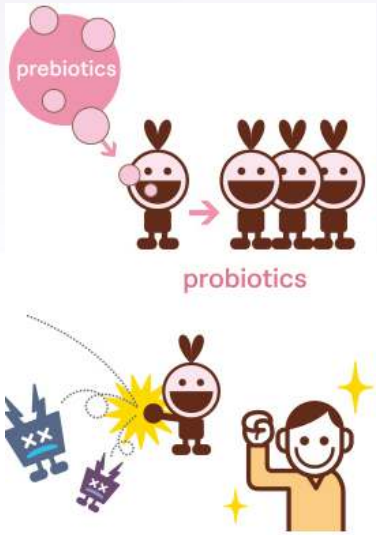


What does the microbiota *do*?

Produces essential nutrients: some vitamins and short-chain fatty acids (SCFA) such as acetate, propionate, butyrate

- SCFA influence pH
- Increased fractional absorption of calcium
- Prebiotic fibers increase bone density/ bone strength in animal models [Weaver CM. Current Osteo Res. 2015;13:125]





Prebiotic Fibers are:

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A lactobacillus party

- Carbohydrates
  - Mainly soluble fibers
- Not digestible (by us)
- Fermentable (by bacteria) → Food for the "good" bacteria

The most common prebiotic fibers used in foods and formulas:

- **Fructo-oligosacharides** (FOS)
- **Galacto-oligosacharides** (GOS)

Systematic Review

- Aim:
  - Primary: conduct meta-analysis and systematic review of literature
  - Secondary: assess other bone indicators (BMC, BTM, Vit D, PTH) to define areas of research

- Results: Meta-analyses of BMD in early-treated patients with PKU
  - **Total BMD Z-score = -0.45** (95% CI -0.61, -0.28)
    - 3 studies; n=133 patients included in analysis
  - **Lumbar spine BMD Z-score = -0.70** (95% CI -0.82, -0.57)
    - 7 studies; n=247 patients included in analysis
  - **Femoral neck BMD Z-score = -0.92** (95% CI -1.10, -0.73)
    - 2 studies; n=78 patients included in analysis

Within nL range for age and sex

- Pooled data: Only one study examined prevalence of BMD below expected range for age (Z-score < -2), appropriate for the population under investigation
  - Prevalence for low BMD for age was 10% in PKU pts vs 2.3% healthy population

Demirdas S et al Rani Singh MGM 2015;114:364 (abstract)

Meta-analysis Conclusions

- Pooled data suggest reduction in BMD Z-score is not clinically important in patients with PKU
  - Mean Z-scores within normal reference range for BMD (Z score > -2)
  - Total BMD and femoral BMD mean Z-scores are above -1
- Results from studies evaluating bone turnover markers (BTM) are inconclusive
- Phenylalanine concentration, vitamin D, PTH, and intake of individual nutrients (calories and protein) do not seem to correlate with BMD or BTM

Demirdas S et al Rani Singh MGM 2015;114:364 (abstract)

Monitoring

- What are optimal biomarkers to monitor? How often? Cost-Benefit?
  - DEXA
  - 25- OH vitamin D
  - Ionized CA, P, Mg
  - Alk Phos? Bone Alk Phos?
  - PTH?
  - Selected bone markers (multiple markers)

Monitoring

Table 2: Comparison of the bone markers between the control and the PKU groups

Parameter	Group	n	Mean (SD)	t-test	*P.* value
Carboxy-terminal propeptide of type I collagen (ng/ml)	Control	42	270.7 (89.6)	0.5	0.5
	Phenylketonuria	33	283.4 (114.7)		
Alkaline phosphatase (U/L)	Control	42	152.0 (43.1)	2.9	0.005
	Phenylketonuria	33	121.6 (46.0)		
Osteocalcin (mg/dl)	Control	42	43.4 (34.5)	6.1	<0.001
	Phenylketonuria	33	13.9 (12.9)		
Receptor activator of nuclear factor κβ ligand (RANKL) (ng/ml)	Control	42	0.1 (0.07)	19.7	<0.001
	Phenylketonuria	33	1.0 (0.2)		
Osteoprotegerin (OPG) (ng/ml)	Control	42	3.3 (2.3)	1.7	0.09
	Phenylketonuria	33	4.0 (0.8)		
Deoxypyridinoline (mmol/mmol creatinine)	Control	42	68.1 (30.7)	4.3	<0.001
	Phenylketonuria	33	32.3 (15.0)		
OPG /RANKL ratio	Control	42	0.56 (0.06)	12	0.09
	Phenylketonuria	33	0.27 (0.13)		

SD: Standard Deviation

Conclusion: total ALP was a good marker for assessment of bone. ALP was negatively correlated with TBMC and BMD-L. Recommend screening pts for ALP; if low then combine with BMD and specific bone markers osteocalcin, RANK-L and Deoxypyridinoline

Koura HM et al. Iran J Pediatr 2014;24:23-28

Conclusions/Practical Considerations

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- Long term elevated plasma PHE concentrations may have partly be associated with compromised bone integrity
- Dietary adherence to medical food may play an important part in bone health
  - Major source of protein, calcium, phosphorus, magnesium, vitamin D
- Vitamin D intake and sun exposure may play a major part in bone status in ALL individuals
  - More studies need to be conducted in PKU patients
- Still not known if patients with PKU are at increased risk of osteopenia
- Unknown: what is the role of increased weight bearing exercise in promoting bone health in PKU patients? Promoting aerobic exercise has positive effect on reducing plasma PHE and increasing PHE tolerance (anecdotal evidence)

Clinical Applications to Support Bone Integrity in Patients with PKU

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1. For optimal bone integrity patients with PKU should do the following:
2. Maintain plasma PHE concentrations in tx range
3. Adhere to life long dietary regimen including consumption of medical food
4. Choose medical foods with variety, taste and form to promote acceptablity
5. Assess and maintain adequate nutrient intakes important for bone integrity (e.g. protein, calcium, magnesium, copper, etc…)
6. Maintain higher BMI (Miras et al 2013; Rani Singh, unreported data 20150
7. Fat intake may be positively affect BMD (unreported data R. Singh 2015)
8. Assure adequate vitamin D intake and status based on new guidelines
9. Non-compliant patients should be counseled to take a multiple vitamin/mineral supplement, as well as a calcium/vitamin D supplement
10. Exercise and avoid negative life style habits

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