Bone Structure and Metabolism

- Bone is formed by osteoblasts that synthesize and secrete the organic matrix.
- Mineralization of the matrix begins soon after its secretion and is completed after several weeks.
- Resorption of bone is carried out by osteoclasts.
- Remodeling of bone continues throughout life.
Osteoclasts erode the bone surface, dissolving mineral and matrix.

Osteoblasts build new bone, laying down collagen and minerals.
Markers of Bone Turnover

Osteoblasts
Bone Formation

Balance

Osteoclasts
Bone Resorption

Osteocalcin
Bone Alk Phosphatase

Pyridinium cross-links
Normal Bone Acquisition

95% of total bone mineral density by age 18 years

Factors that Affect Bone Mineral Density
- Nutrition
  - Ca/Vit D/Mg/Protein
  - Mn/Cu/Zn
- Exercise (weight bearing)
- Genetics
- Lifestyle
  - Smoking, alcohol
Osteopenia and Osteoporosis

Definitions:

Osteopenia: - 1.5 - 2.5 DEXA Z score [>1 SD but < 2.5 SD below peak adult BMC/BMD]

Osteoporosis: < 2.5 DEXA Z score [≥ 2.5 SD below peak young adult]
Increased Ca intake through adolescence can decrease risk of hip fractures up to 50%
PHENYLKETONURIA: How is this different from Non-PKU individuals

• #1: Phenylketonuria is a lifelong chronic autosomal recessive disorder of amino acid metabolism

• #2: Lifelong treatment consists in replacement of natural 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 synthetic sources

• Patients with PKU do not have access to a variety of foods which can affect bone status

• 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
PHENYLKETONURIA

- PKU patients are at risk for fracture esp. over 8 years of age (Greeves, *Acta Paediatr*, 1997)
- PKU not the only metabolic disorder with increased risk fractures (GSD I, Galactosemia, Organic Acidemias)
- Why the increased risk in metabolic disorders?
  - Pathogenesis has not been elucidated
  - Use of synthetic diet as major source of nutrients, including calcium, vitamin D, phosphorus and protein may be an issue
  - Dietary non-compliance (reduced bone-nutrient intakes)
  - Reduced intake of bone forming nutrients
  - Chronic elevated plasma PHE concentrations over age 12 years
Published studies on bone mineral density and PKU

- Carson, (1990) *Pediatr Radiology*
- J. Zeman (1999) *Acta Paediatr*
- D. Modan-Moses (2007) *J Inherit Metab Dis*
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

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
- Diet non-compliance leads to poor bone status

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

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.
Pyridinium Cross-Links

![Diagram showing the process of collagen maturation involving lysine hydroxylation and oxidation, resulting in pyridinium and deoxypyridinoline cross-links.](image-url)
Values are mean ± SEM.

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

**Ca Intake, mg/day**

- 0
- 500
- 1000
- 1500
- 2000

**Age, Years**

- 2
- 4
- 6
- 8
- 10
- 12

**Ca Intake, mg/day RDA**

- 350
- 345
- 309
- 315
- 362

**Maximum reference range**

**Alkaline Phosphatase, U/L**

- 0
- 50
- 100
- 150
- 200
- 250
- 300
- 350

**Age, Years**

- 2
- 4
- 6
- 8
- 10
- 12

**Maximum reference range**
Plasma Calcium and Phosphorus Concentrations in PKU

**Calcium**
- mg/dL
- Normal range: 9-12 mg/dL
- Data for ages 0 to 12 years

**Phosphorus**
- mg/dL
- Normal range: 4-5 mg/dL
- Data for ages 0 to 12 years
**PYRIDINOLINE**

* p < 0.01

**DEOXYPYRIDINOLINE**

* p < 0.01
CORRELATION OF URINARY DEOXYPYRIDINOLINE WITH CALCIUM INTAKE

\[
r^2 = 0.70 \\
p < 0.01
\]
CONCLUSIONS

• Bone resorption is significantly higher than normal controls in patients with PKU after 8 years of age, suggesting increased bone turnover.

• Increased dietary CA intake was correlated with decreased bone resorption. Higher Ca = Less resorption

• Compliance with diet decreases before changes in urinary pyridininium cross-links, suggesting a causal relationship.
PKU Mice Bone Study Design

54- weanling PKU and Control Mice

56 day treatment

Blood bone measures: osteocalcin, Pyr crosslinks

Bone studies: Femur strength, BMD, BMC

Yannicelli and Medieros, 2002
Femur and Tibia Weight

Yannicelli and Medieros, 2002
Urinary Pyridinium Excretion

Yannicelli and Medieros, 2002
Conclusions from PKU Mouse Study

• Moderate protein restriction did not significantly affect bone integrity
• Hyperphe adversely affected femur length and total femur area in untreated PKU mice
• Treated PKU mice have bone status similar to control mice
• Plasma PHE conc. were positively correlated with DPD/creatinine excretion
Hypothesis on Hyperphenylalaninemia and decreased bone mineral content

- Increased plasma PHE results in production of phenylketones (increased acid load)
  - Bone must resorb minerals to counter increase acid load

- Some amino acid based formulas can increase renal net acid excretion and decreased urine PH.
  - May be based on high sulfur and chloride content
  - Further studies (Nord et al 1988. J Inherit Metab Dis) did not find similar results
Bone mineral status among PKU patients is one of the interest of Polish PKU Working Group (2003-2008)

- 195 Caucasian subjects with classical PKU on dietary treatment (mean age: 13.7 ± 0.6 y)
- Collaborative study from 8 medical universities in Poland
- Measurements:
  - Total and Lumbar Spine bone mineral density (BMD) was assessed using dual energy X-ray absorptiometry (DXA)
  - Biochemical indices as: serum CA/P, parathyroid hormone, ALK PHOS and 24h urine calcium were determined
- Retrospective records of blood phenylalanine levels were evaluated in 57 PKU patients over 14 years (14-28 y)

Reported as poster at ICIEM, San Diego 2009
Mean Z-score BMD decreased with age and was significantly lower among young adults.

Mean value of TOTAL BODY Z-score

BMD – Lumbar Spine Z-score

Conclusions/Practical Considerations

• Pathogenesis is still not known. Probably multifactorial.
• 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)
Monitoring

• Major question: what are you going to do with the data once you collect it?
• What are optimal biomarkers to monitor? How often? Cost-Benefit?
  – DEXA
  – 25- OH vitamin D
  – Ionized CA, P, Mg
  – PTH?
  – Plasma amino acids?
Clinical Applications to Support Bone Integrity in Patients with PKU

• For optimal bone integrity patients with PKU should do the following:
  – Maintain plasma PHE concentrations in tx range
  – Adhere to life long dietary regimen including consumption of medical food
    • Choose medical foods with variety, taste and form to promote acceptability
  – Assess and maintain adequate nutrient intakes important for bone integrity (e.g. protein, calcium, magnesium, copper, etc…)
  – Assure adequate vitamin D intake and status based on new guidelines
  – Non-compliant patients should be counseled to take a multiple vitamin/mineral supplement, as well as a calcium/vitamin D supplement
Gracias

Obrigado!