

**Acid**

**Base**

**SYMPOSIUM**

**2<sup>nd</sup> International  
Acid-Base Symposium  
Nutrition – Health – Disease**

Munich, Germany, September 8 – 9, 2006

**Final Program & Abstracts**



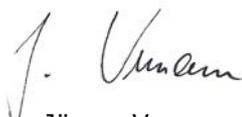
## INVITATION

The 2<sup>nd</sup> International Acid-Base Symposium intends to confirm the essential role acid-base homeostasis plays for human health and the beneficial disease-modifying aspects of an adequate dietary base supply.

To what extent the diet can affect the acid–base homeostasis has been the subject of controversy in the past. Based on new scientific findings, causal evidence has been furnished for the positive effects of a well-balanced acid–base equilibrium. Although diet-induced latent acidosis does not produce major changes in the blood pH, the compensation mechanisms of the kidney inevitably lead to the consumption of endogenous buffer reserves and, therefore, predominantly to a loss of bone substance if the increased acidification caused by a surplus of animal protein and a shortage of plant alkaline substances in the diet persists. A disturbance of the muscle protein metabolism as well as of the structure and function of cartilage are other negative consequences of the endogenous compensation, which aggravate degenerative diseases such as arthrosis or rheumatism.

This 2<sup>nd</sup> International Symposium again offers the opportunity to review several research fields on acid-base homeostasis and to discuss among specialists recent data and new ideas. Additionally the beautiful venue of Munich contributes to make this symposium a stimulating and agreeable event, rich in personal contacts.

Munich, September 2006



Jürgen Vormann



Thomas Remer



Thomas Goedecke

## COMMITTEE

Prof. Dr. Jürgen Vormann,  
Institute for Prevention and Nutrition (IPEV)  
85737 Ismaning, Germany

Prof. Dr. Thomas Remer,  
Research Institute of Child Nutrition (FKE)  
44225 Dortmund, Germany

Dr. Thomas Goedecke,  
Protina Pharmaceuticals  
85737 Ismaning, Germany

**PROGRAM****FRIDAY, September 8, 2006**

- 8.15 – 8.30**                    **Opening**  
J. VORMANN, Institute for Prevention and Nutrition, Ismaning, DE  
T. REMER, Research Institute of Child Nutrition, Dortmund, DE
- 8.30 – 8.55**                    **Acid-base dietary components, bone mineral density, and fracture risk in the Framingham Osteoporosis Study.**  
K. L. TUCKER, Tufts University, Boston, US
- 8.55 – 9.20**                    **PRAL-independent diet effects on NEAP: acid base considerations in stone-age sweet potato eaters, modern-day sweet potato eaters, and high-protein consumers.**  
T. REMER, Research Institute of Child Nutrition, Dortmund, DE
- 9.20 – 9.45**                    **Food composition and acid-base balance: alimentary acid load and clinical implications in neonates.**  
H. KALHOFF, Clinical Centre Dortmund, DE
- 9.45 – 10.05                    Coffee break
- 10.05 – 10.30**                    **Metabolic acidosis induces bone resorption via proton receptor-mediated activation of inositol phosphate-dependent calcium signaling.**  
D. A. BUSHINSKY, University of Rochester School of Medicine, US
- 10.30 – 10.55**                    **Regulation of bone cell function by extracellular pH.**  
T. R. ARNETT, University College London, UK
- 10.55 – 11.20**                    **Does the skeleton play a role in acid-base homeostasis? Current evidence: future perspectives.**  
S. A. LANHAM-NEW, University of Surrey, UK
- 11.20 – 11.45**                    **Dietary indicators of acid base balance and bone accrual in pubertal children.**  
F. TYLAVSKY, University of Tennessee, US
- 11.45 – 13.00                    Lunch
- 13.00 – 13.25**                    **Is acid-base balance important for bone health in postmenopausal women? Evidence from cohort and intervention studies in Aberdeen.**  
H. M. MACDONALD, University of Aberdeen, UK
- 13.25 – 13.50**                    **The effect of bicarbonate in mineral water on bone metabolism: interventional studies.**  
P. BURGHARDT, Clinique Bois Cerf/Hirslanden, Lausanne, CH

- 13.50 – 14.15**                    **Ovariectomy and dietary induced metabolic acidosis both result in decreased bone quality through different mechanisms: experience with an ovine model.**  
J. M. MACLEAY, Colorado State University, Fort Collins, US
- 14.15 – 14.30**                    **The association of dietary acidity with bone mineral density in postmenopausal women.**  
A. C. HARDCASTLE, University of Aberdeen, UK
- 14.30 – 14.50                    Coffee break
- 14.50 – 15.15**                    **Could minor cations and anions or other constituents such as fibres, contribute to the alkalinising properties of plant foods ?**  
C. DEMIGNÉ, Institut National de la Recherche Agronomique, Clermont-Ferrand/Theix, FR
- 15.15 – 15.30**                    **Estimation of the dietary acid generating potential of the elderly British population: analysis of the National Diet and Nutrition Survey (NDNS) adults aged 65 years and over using estimates of net acid excretion indirect (NAE<sub>ind</sub>) and net rate of endogenous non-carbonic acid production (NEAP).**  
R. H. T. GANNON, University of Surrey, UK
- 15.30 – 15.45**                    **Potential renal acid load in the diet of German children and adolescents: impact of food groups and trends.**  
U. ALEXY, Research Institute of Child Nutrition, Dortmund, DE
- 15.45 – 16.00**                    **Provision of dietary alkali in the UK diet: a pilot study to examine the effects of supplementation with 'Horlicks' on estimates of potential renal acid load (PRAL) and net acid excretion indirect (NAE<sub>Indirect</sub>) in postmenopausal women.**  
J. CATTERICK, University of Surrey, UK
- 16.00 – 16.15**                    **Acid-base balance in vegetarians and non-vegetarians.**  
P. CLARYS, Vrije Universiteit Brussel, BE
- 16.15 – 16.35                    Coffee break
- 16.35 – 17.00**                    **Mild acidosis destabilizes human transthyretin and may increase the risk for amyloidosis.**  
K. ALTLAND, Justus-Liebig-University of Giessen, DE
- 17.00 – 17.25**                    **Alkaline mineral supplementation decreased pain in rheumatoid arthritis: a randomized, controlled study.**  
R. M. CSEUZ, Rivita Clinic Budapest, HU
- 17.25 – 17.50**                    **Drinking water composition and disease - is acidity a key factor?**  
R. RYLANDER, Biofact Environmental Health Research Centre, Ledum, SE
- 19.30                                Conference Dinner

**SATURDAY, September 9, 2006**

- 8.30 - 8.55**                    **Dietary NaCl induces low-grade hyperchloremic metabolic acidosis in healthy humans.**  
L. A. FRASSETTO, University of California, San Francisco, US
- 8.55 - 9.20**                    **The effect of sodium acetate ingestion on acid-base balance and resting metabolism in man.**  
D. BALL, Defence Science Technology Laboratory, Fareham, UK
- 9.20 - 9.45**                    **Osmotically inactive sodium retention is correlated with low-grade metabolic acidosis.**  
M. HEER, German Aerospace Centre, Cologne, DE
- 9.45 - 10.00**                **Bone loss because of high sodium intake: is there a connection to the acid-base balance?**  
P. FRINGS, German Aerospace Centre, Cologne, DE
- 10.00 - 10.20                Coffee break
- 10.20 - 10.45**                **Urinary pH is an indicator of dietary acid-base load in a population: results from the EPIC-Norfolk cohort study.**  
A. WELCH, University of Cambridge, UK
- 10.45 - 11.10**                **How important is dietary acid-base balance for bone health in different age groups?**  
F. GINTY, Medical Research Centre, Cambridge, UK
- 11.10 - 11.35**                **Food composition and acid-base balance: experimental observations on alimentary alkali depletion in herbivores.**  
H. KIWULL-SCHÖNE, Ruhr University Bochum, DE
- 11.35 - 12.00**                **Lactic acid is not the source of protons in metabolic acidosis.**  
R. A. ROBERGS, University of New Mexico, Albuquerque, US
- 12.00 - 12.15**                **The renal net acid excretion capacity across various age-groups.**  
S. BERKEMEYER, Research Institute of Child Nutrition, Dortmund, DE
- 12.15 - 12.30**                **Associations of biomarkers of acid load and cortisol secretion with potentially bioactive free glucocorticoids in healthy lean adults: a pilot study.**  
T. REMER, Research Institute of Child Nutrition, Dortmund, DE
- 13.00                            End of symposium/Excursion

**SOCIAL PROGRAM****FRIDAY, September 8, 2006****19.30 Conference Dinner**

Paulaner am Nockherberg  
Hochstraße 77  
81541 München

Conference Dinner is included in the registration fee.

**SATURDAY, September 9, 2006****Excursion to Lake Starnberg**

Lake Starnberg is situated 20 km in the South of the Bavarian capital in front of the majestic scenery of the Alps with stunning mountain views. No other Bavarian area boasts so many old castles and stately homes fringing the watersides of Lake Starnberg.

**13.00 Bus transfer to Starnberg** (Bus departure at main entrance Dorint Novotel)**14.45 Boat Trip to the Buchheim Museum, Bernried**

Buchheim Museum is located directly at the banks of Lake Starnberg. The visit comprises an English guided tour of the 'Buchheim Collection – Museum of Imagination' encompassing an extraordinarily wide spectrum of outstanding Expressionist art. The collections of Lothar-Günther Buchheim are embedded in an architecture of extraordinary diversity. A unique architectural feature is the deck that is suspended twelve-meters high over the lake, providing museum visitors with an overwhelming view of the town of Starnberg and the Alps.

**18.30 Dinner**

Forsthaus am See  
Am See 1  
82343 Possenhofen

**22.00 Return to Conference Hotel**

Excursion fee is 50 € per person including bus transfer, boat trip, guided museum tour and dinner. Please register at the Congress Secretariat.

## MAIN TALKS

Klaus Altland, Germany  
Timothy R. Arnett, United Kingdom  
Derek Ball, United Kingdom  
Peter Burckhardt, Switzerland  
David A. Bushinsky, United States  
Regina M. Cseuz, Hungary  
Christian Demigné, France  
Lynda A. Frassetto, United States  
Fiona Ginty, United Kingdom  
Martina Heer, Germany  
Hermann Kalhoff, Germany  
Heidrun Kiwull-Schöne, Germany  
Susan A. Lanham-New, United Kingdom  
Helen M. MacDonald, United Kingdom  
Jennifer M. MacLeay, United States  
Thomas Remer, Germany  
Robert A. Robergs, United States  
Ragnar Rylander, Sweden  
Katherine L. Tucker, United States  
Francis A. Tylavsky, United States  
Ailsa Welch, United Kingdom

## SHORT LECTURES

Ute Alexy, Germany  
Shoma Berkemeyer, Germany  
Janet Catterick, United Kingdom  
Peter Clarys, Belgium  
Petra Frings, Germany  
Richard H. T. Gannon, United Kingdom  
Antonia C. Hardcastle, United Kingdom

## **Potential renal acid load in the diet of German children and adolescents: impact of food groups and trends.**

UTE ALEXY, THOMAS REMER

Research Institute of Child Nutrition, Dortmund, Germany

**Introduction:** Although the impact of acid-base status on health is widely accepted, only few data on the dietary acid load in healthy people, especially children, are available. Here, we describe the PRAL of the diet of German children and adolescents from the DONALD (Dortmund Nutritional and Anthropometric Longitudinally Designed) Study, the impact of nutrients, food groups, age and time trends on PRAL.

**Methods:** 4710 three-day dietary records from 720 study participants (351 boys, 369 girls) aged 3-18 years, collected between 1995-2005, were analysed. PRAL (mEq/day, mEq/MJ/day) was calculated using a published algorithm including dietary protein, phosphorus, magnesium and potassium. Age and time trends were estimated using the SAS-procedure PROC MIXED.

**Results:** PRAL was positive in all age-groups ranging between 5.9-20.5 mEq/day, with higher values in boys > 8 years, even after adjustment for energy intake. PRAL (mEq/day and mEq/MJ) increased with age in younger children (both sexes, 3-7 years) and boys (8-18 years). Food groups with the highest impact on daily diet acidity were meat-fish-eggs and grain-bread. Fruit-juices and vegetables yielded the highest dietary alkalinity (average of all age-groups). While percentage energy from fat decreased during the study period ( $p < 0.001$ ), no time trends were found for PRAL.

**Discussion:** Present dietary habits in children and adolescents with low intakes of cheese and higher intakes of fruit compared to vegetables, are responsible for the observed contributions of the food groups on acid base status. In this respect, boys should be encouraged to eat more base-forming foods. PRAL could have a valuable role to play along with other indices in the evaluation of dietary quality.

## Mild acidosis destabilizes human transthyretin and may increase the risk for amyloidosis.

KLAUS ALTLAND

Institute of Human Genetics, Justus-Liebig-University, Giessen, Germany

The transthyretin type of familial amyloidosis, produced by mutations in the transthyretin (TTR) gene, is an autosomal dominant disease that first manifests itself in the adult. The more than 90 so-called amyloidogenic mutations lead to destabilization of TTR that is formed in the liver and secreted into the bloodstream, and eventually results in extracellular deposits of insoluble amyloid on nerves and muscle fibers of the heart and blood vessels. In familial amyloid polyneuropathy (FAP) peripheral nerves are primarily affected. Deposits in the arterial vessel wall and in the heart muscle are the primary characteristics of familial amyloid cardiomyopathy (FAC). In senile systemic amyloidosis (SSA), it is primarily the heart and vasculature that are affected. There are no TTR mutations in this condition.

The only therapy is liver transplantation for FAP or heart and liver transplantation for FAC. There is no therapy for SSA. The disease is seen very rarely in children, and in patients of German ancestry it rarely begins before the age of 40. It can be concluded that besides genetic predisposition, age plays a considerable role. We cannot help growing old, but we can influence the condition of the organs/tissues on whose functioning our quality of life and our lifespan depend.

Human TTR is mainly synthesized in the liver, *plexus choroideus* and retina. A single gene codes for the 127 amino acids of the TTR monomer. Two monomers are rather firmly bound as a dimer and two dimers are bound as a tetramer with binding sites for thyroxine inside a channel and for retinol binding protein (RBP) at the outer surface. Mutant amyloidogenic TTR has shown to be conformationally unstable. Here, we demonstrate that dimers from normal TTR monomers decay into monomers within the pH range 7.0 – 6.5 and, that monomers with the most frequent amyloidogenic TTR-Val30Met mutation have an increased risk to decay into monomers at pH levels of mild interstitial acidosis, i.e. pH 7–7.4. We present arguments favouring the hypothesis that a hydrogen bridge between the NE2 nitrogen of His 31 and the hydroxyl oxygen of Ser46 is a vulnerable structure of normal TTR and specifically of TTR-Val30Met for changes of pH. It is postulated that trials to protect against long lasting episodes of interstitial acidosis could help to protect against early onset of amyloidosis and to inhibit the progression of the disease.

## Regulation of bone cell function by extracellular pH.

TIMOTHY R. ARNETT

Department of Anatomy and Developmental Biology, University College London, United Kingdom

Elimination or buffering of the acid produced as a result of metabolism poses a fundamental problem for all multicellular organisms. The most basic function of the vasculature is to deliver nutrients and O<sub>2</sub> to cells and to remove waste products, including H<sup>+</sup> and CO<sub>2</sub>, which, in land vertebrates, are excreted via urine and expired air. The skeletons of land vertebrates contain a massive reserve of base, which is ultimately available as a 'failsafe' mechanism to buffer H<sup>+</sup> if the kidneys and lungs are unable to maintain acid-base balance within narrow limits. The classic cause of chronic, systemic acidosis is kidney disease, and this is associated with bone loss. Mild chronic acidosis often occurs as a result of ageing or menopause or because of dietary acid ingestion. Acute, severe systemic acidosis can be caused by gastroenteritis, where it is associated with increases in bone resorption indices; acute, severe acidosis is also readily induced by vigorous exercise. Acidosis can arise locally (at tissue level) as a result of reduced vascular supply due to inflammation, infection, tumours, wounds, diabetes, ageing, or simply as a result of increased cellular metabolism (and thus H<sup>+</sup> production) due to the stimulatory, mitogenic action of growth factors and cytokines.

The deleterious action of systemic acidosis on the skeleton has long been known but was generally thought to result simply from physico-chemical dissolution of bone mineral - ie, that the skeleton acts as a 'giant ion exchange column' to passively buffer systemic H<sup>+</sup>. However, it is now clear that this net buffering effect is cell-mediated rather than physico-chemical in nature. Bone resorption by cultured osteoclasts from all species studied to date is stimulated directly by H<sup>+</sup> ions. Osteoclasts are particularly sensitive to [H<sup>+</sup>] between pH 7.1-7.3, such that pH reductions of only a few hundredths of a unit cause a doubling of resorption pit formation. Below pH ~7.0, the stimulatory effect plateaus, whereas above pH 7.4, resorption is 'switched off'. Similar responses occur in calvarial bone organ cultures; moreover, H<sup>+</sup>-stimulated Ca<sup>2+</sup> release is almost entirely osteoclast-mediated, with a negligible physico-chemical component. Acidification is the key initial requirement for osteoclasts to be able to excavate resorption pits; once activated, osteoclasts can be further stimulated by a wide range of agents including parathyroid hormone, 1,25(OH)<sub>2</sub> vitamin D, ATP, ADP and RANK ligand. Thus, extracellular H<sup>+</sup> may be regarded as the long-sought 'osteoclast activation factor'. We recently made the surprising observation that osteoclastic resorption can also be activated (at alkaline pH) by low nanomolar concentrations of the alkaloid, capsaicin. The capsaicin receptor (TRPV1 or VR1) is a cation channel that is also activated by low pH and heat, and thus is a candidate receptor that could mediate osteoclast activation.

Acidosis also affects osteoblast function adversely. The mineralisation of bone matrix nodules deposited by osteoblasts in long-term culture is progressively blocked as pH is reduced, with complete inhibition at pH 6.9. This appears to be due not only to a marked increase in bone mineral solubility at low pH but also to strong inhibition of the expression/activity of osteoblast alkaline phosphatase (required for mineralisation); however, osteoblast growth and collagen production are unaltered at pH values as low as 6.9. These results may help explain the osteomalacia that can occur in chronic acidosis.

Considered together, these results indicate that a remarkable reciprocal relationship exists between osteoclast activation and matrix mineralisation by osteoblasts over the pH range ~7.4 to ~6.9. Thus, acidosis exerts a major 'double-negative' action on bone turnover/maintenance. Drugs that block acid-sensing receptors or shift acid-base balance in the alkaline direction may provide novel therapies for bone loss disorders.

## The effect of sodium acetate ingestion on acid-base balance and resting metabolism in man.

DEREK BALL<sup>1,2</sup>, GORDON I. SMITH<sup>1</sup>

<sup>1</sup>University of Aberdeen, United Kingdom; <sup>2</sup>Defence Science and Technology Laboratories, Fareham, United Kingdom

The consumption of a high-protein diet has been shown to have an acidifying effect on blood acid-base status, primarily due to the metabolism of sulphur containing amino acids (Greenhaff et al., 1988). In contrast, it has been clearly established that long-term adherence to a vegetarian diet has an alkalinising effect on urinary acid-base status (Ball and Maughan, 1997). While the ingestion of the sodium salts of weak organic acids has been shown to produce a mild metabolic alkalosis the metabolic consequences of the changes in acid-base status have been relatively ignored (Ball and Maughan, 1993). We hypothesised that the administration of the sodium salt of acetic acid would suppress fat metabolism despite the favourable shift in acid-base balance towards increasing lipolysis. In two separate studies we have investigated the effect of sodium acetate ingestion on acid-base balance and resting metabolism. In the first study 6 healthy individuals volunteered to ingest a bolus dose (2 mmol/kg body mass) of either sodium acetate or sodium citrate and over the following 90 min the changes in acid-base status were measured using arterialised-venous blood samples and substrate utilisation from samples of expired air. In a second study the use of labelled <sup>13</sup>C acetate provided a means of calculating the amount of ingested acetate that was oxidised at rest. In this study 8 healthy volunteers ingested either sodium acetate or sodium bicarbonate (NaHCO<sub>3</sub>) at a dose of 2 mmol/kg b.m. and over the following 180 min the acid-base and metabolic effects of ingesting the sodium salts were measured. In both studies we found that the ingestion of the sodium salts induced a mild metabolic alkalosis. However, dependent upon the sodium salt that was administered the metabolic effect differed significantly. Ingestion of sodium citrate had no effect on resting substrate utilisation but following sodium acetate ingestion (2 mmol/kg b.m) there was a 30% decrease in fat utilisation that appeared to be accounted for by the oxidation of acetate. The ingestion of an equimolar dose of NaHCO<sub>3</sub> induced a metabolic alkalosis which had the effect of increasing fat utilisation. Using the <sup>13</sup>C labelled acetate it was found that 80% of ingested acetate was oxidised over the 180 min period but in contrast to the first study there was no significant effect of acetate ingestion on fat oxidation when compared with the pre-ingestion value. Fat utilisation was, however, significantly lower in the sodium acetate trial when compared to that following NaHCO<sub>3</sub> ingestion.

### References

- Ball, D., Maughan. R.J. (1993) Proc Nutr Soc. 52:266A  
Ball, D., Maughan. R.J. (1997) British J Nutr. 78:683-693.  
Greenhaff, P.L., Gleeson, M., Maughan R.J. (1988). Eur J Appl Physiol. 57:583-590.

### **The renal net acid excretion capacity across various age-groups.**

SHOMA BERKEMEYER<sup>1</sup>, JÜRGEN VORMANN<sup>2</sup>, RAGNAR RYLANDER<sup>3</sup>, THOMAS REMER<sup>1</sup>

<sup>1</sup>Research Institute of Child Nutrition, Dortmund, Germany; <sup>2</sup>Institute for Prevention and Nutrition, Ismaning, Germany; <sup>3</sup>Environmental Medicine, University of Gothenburg, Sweden

**Background:** There are clear indications that systemic bicarbonate buffer decreases and free proton increases with age. It is believed this is caused by decrease in renal function. However, till date, it has never been shown that renal net acid excretion (NAE) capacity actually falls from young to old age.

**Objective:** To find out if NAE capacity (NAEC) is lower in elderly compared to young adults, adolescents and prepubescents.

**Design:** Anthropometric data and 24-h urinary pHs and NAEs were determined in healthy, subject groups: elderly (55-75y;  $n=85$ ), young adults (18-22y;  $n=109$ ), adolescents (13-14y;  $n=169$ ), and prepubescents (6-7y;  $n=254$ ), latter three samples selected from the DONALD-Study. NAEC was determined by the residues of net acid excretion (NAE) on urinary pH. Regression analyses were run using SAS with sex and age-groups as dummy variables.

**Results:** 24-h urinary pH and NAE (5.94, 60.03mEq/d) in the elderly were significantly lower ( $P<0.05$ ) and higher ( $P<0.05$ ), respectively, compared to the three other groups'. Multiple regression analyses with NAEC as outcome revealed a significant negative, though modest, association for the elderly age dummy ( $P<0.01$ ) and an almost significant positive association for the prepubescents age-group ( $P=0.06$ ).

**Conclusions:** We report for the first time using kidney excretion parameters that NAEC is lower in healthy elderly than in young healthy adults evenso this decrease contributes only modestly to the explained variation of NAEC across age-groups.

## The effect of bicarbonate in mineral water on bone metabolism: interventional studies.

PETER BURCKHARDT

Clinique Bois Cerf/Hirslanden, Lausanne, Switzerland

Oral K bicarbonate improved calcium balance in postmenopausal women (Sebastian 1994). We could show an effect of bicarbonate (Bic) in food and in mineral water, but not of an acid load, on bone metabolism in 4 controlled intervention trials.

A) Basic food and Bic-rich mineral water were given to 8 male volunteers during 4 days, and then replaced by a control diet, equal in calories, protein, Na, and calcium (Ca), in a randomized order after a washout period. The first diet decreased sign. urinary Ca excretion by 45%, and resorption markers (CTX/creat.) by 15% (Buclin 2001). Therefore, short term nutritional alkali-load reduced bone resorption.

B) An oral acid load of 6.4 g (120 mmol) NH<sub>4</sub>Cl per day for 2 days given to 8 volunteers, and compared to 120 nmol NaCl/day, lowered urin. pH by about 1 unit over the whole day compared to NaCl (p=0.0001), and slightly decreased blood pH and bicarbonate by about 2 mmol/l (p=0.002, resp. 0.0001). But after 1 day it had no influence on markers of bone metabolism or on their response to Ca (Buclin 2003).

C) Bic-rich mineral water in 20 healthy (Bic 2643 mg and Ca 378 mg/1.5 l) was given over 4 weeks to 10 young women on a free diet and compared with a Ca-rich water (Bic 605 mg, Ca 728 mg in 1.5 l) (n=10). The Bic-rich water increased slightly urine pH (fasting and 24 hrs), and lowered sign. the resorption markers (urin. CTX/creat. in 24 hr urine) by 25% (ANOVA p<0.05). The Ca-rich water increased only urinary Ca excretion by 31% (Burckhardt).

D) The effect in Calcium sufficiency: 30 young women on an equilibrated free diet were given 1.5 l per day of either a Ca-rich water (Bic 437 mg, Ca 780 mg/1.5 l) or of a water rich in both Ca and Bic (Bic 3258 mg, Ca 821 mg/1.5 l). While the Ca-water only had random effects on bone resorption, the Bic-Ca-water sign. decreased PTH by 16% and CTX by 15% (Wynn 2006, in prep).

Conclusion: Mineral water rich in bicarbonate, but not mineral water rich in calcium, decreases bone resorption in normal subjects, even on a free diet and a high calcium intake (> 1500 mg/d).

### References

- Sebastian et al. (1994) N Engl J Med 330:1776-1781.  
Buclin T et al (2001) Ost Int 12 : 493-499  
Buclin T (2003), abstr. ISNAO 2003; unpublished.  
Burckhardt P et al (2002) JBMR; 17(suppl) S476-M360.

## Metabolic acidosis induces bone resorption via proton receptor-mediated activation of inositol phosphate-dependent calcium signaling.

NANCY S. KRIEGER, KEVIN K. FRICK, KEITH NEHRKE, DAVID A. BUSHINSKY  
University of Rochester School of Medicine, United States

Metabolic acidosis (Met) increases urine calcium (Ca) excretion without an increase in intestinal Ca absorption, resulting in net loss of bone mineral. In vitro, Met stimulates net Ca efflux from neonatal mouse calvariae by stimulation of a prostaglandin E<sub>2</sub>-dependent increase in RANKL, leading to osteoclastic bone resorption. However, the pathway by which increased extracellular [H<sup>+</sup>] transduces an intracellular signal is not clear. G protein-coupled proton sensing receptors (PSRs) provide a potential mechanism for transduction of extracellular acidosis into intracellular responses. Transcripts for the 4 known PSRs, OGR1, GPR4, TDAG8, and G2A, are detectable in total RNA isolated from cultured calvariae. To determine if OGR1, which is coupled to inositol phosphate (IP), modulates Met-stimulated bone Ca efflux, we utilized the OGR1 inhibitor CuCl<sub>2</sub> (100 μM) and found that it significantly inhibited Met-induced net Ca efflux from calvariae. Increased intracellular metabolites of IP lead to an increase in intracellular Ca (Ca<sub>i</sub>). We measured Ca<sub>i</sub> by fluorescent imaging of fura-loaded primary bone cells. The cells were analyzed in a closed chamber with entry and exit ports to facilitate rapid medium change at a fixed pH, Pco<sub>2</sub> and [HCO<sub>3</sub><sup>-</sup>]. Infusion of physiologic Met medium (pH = 7.11, Pco<sub>2</sub> = 45 mmHg, [HCO<sub>3</sub><sup>-</sup>] = 14 mM) induced a marked, rapid, flow-independent, transient increase in Ca<sub>i</sub> in individual cells, which was inhibited by CuCl<sub>2</sub>. We then tested the effect on bone resorption of several inhibitors that block different steps within the IP<sub>3</sub> pathway: 2-aminoethoxydiphenyl borate (2-APB), which inhibits IP<sub>3</sub> receptors and the subsequent increase in Ca<sub>i</sub>; thapsigargin (TG) an ER Ca-ATPase inhibitor which depletes Ca<sub>i</sub> stores; and TMB-8, which blocks Ca release from ER. Neonatal mouse calvariae were incubated for 48h in Met (pH ~7.11) or neutral (Ntl, pH ~7.40) medium in the absence or presence of each of these inhibitors (inh). Medium was changed at 24 h. All three inhibitors significantly decreased the net Ca efflux which was induced by incubation in Met at 24-48h.

Inhibitor	Conc	Ntl	Met	Ntl + inh	Met + inh
APB	100 μM	131 ± 55	670 ± 101*	9 ± 38 <sup>+</sup>	264 ± 138 <sup>+</sup>
TG	100 μM	174 ± 45	866 ± 103*	122 ± 32 <sup>+</sup>	290 ± 25 <sup>*+0</sup>
TMB-8	100 μM	326 ± 68	933 ± 62*	1 ± 26 <sup>*+</sup>	319 ± 80 <sup>+0</sup>

Data are nmoles Ca released/bone/24 hr (mean ± SE); n =6-8 for each group; \* p< 0.05 vs Ntl; <sup>+</sup> p< 0.05 vs Met; <sup>0</sup> p< 0.05 vs Ntl + inh.

These results are consistent with Met activation of OGR1 to induce IP<sub>3</sub>-dependent Ca<sub>i</sub> transients, which may then modulate osteoblastic activity and lead to the subsequent increase in osteoclastic bone resorption.

**Provision of dietary alkali in the UK diet: a pilot study to examine the effects of supplementation with 'Horlicks' on estimates of potential renal acid load (PRAL) and net acid excretion indirect (NAE<sub>Indirect</sub>) in postmenopausal women.**

JANET CATTERICK<sup>1</sup>, RICHARD H.T. GANNON<sup>1</sup>, DAVID.P. LOVELL<sup>2</sup>,  
HELEN M. MACDONALD<sup>3</sup>, D. JOE. MILLWARD<sup>1</sup>, SUSAN A. LANHAM-NEW<sup>1</sup>

<sup>1</sup>Centre for Nutrition & Food Safety, School of Biomedical & Molecular Sciences, University of Surrey, United Kingdom; <sup>2</sup>Postgraduate Medical School, University of Surrey, United Kingdom; <sup>3</sup>Department of Medicine & Therapeutics, University of Aberdeen, United Kingdom

Acid-base homeostasis is critical to overall health. In recent years, there has been a particular focus on the role of the dietary alkali supply on bone. Dietary acidity is particularly influenced by the level of PRAL (potential renal acid load) which is a predictor of the dietary component of net acid excretion (NAE) (Remer et al., 2003). In a previous study, we have shown that hot beverages containing potassium bicarbonate have a substantive negative PRAL and hence are suppliers of dietary alkali. (Catterick et al., 2006).

The aim of this pilot study was to compare estimates of dietary acidity (using PRAL and NAE<sub>Indirect</sub>) associated with the habitual diets of postmenopausal women when drinking 'Horlicks' once a day and when not drinking Horlicks. The period of investigation was 7 days. Postmenopausal women aged 55-64 years (n 12) were recruited. All women were of Caucasian origin and were in full time employment. Subjects were randomly recruited into two groups and were asked to complete 7-d food diaries for two consecutive weeks. The first group of 6 women were asked to follow their normal diet during week 1 and then to include a standard portion of 'Horlicks Light' drink every day for 7d during week 2. The second group of 6 women followed the routine above but in reverse order. The food diaries were coded and analysed using the Win-Diet computer programme (2005). PRAL and NAE<sub>Indirect</sub> were calculated using the appropriate formulae.

Analysis of the food diaries for 12 post menopausal women aged 55-64 years revealed that for 9 subjects, the PRAL<sub>full</sub> value was lower for the week when the subject was consuming one 27 g portion of Horlicks Light once a day, with a mean value of PRAL<sub>full</sub> for the non-Horlicks week of -2.8 mEq (SD 7.0), and a mean for the Horlicks week of -10.5 mEq (SD 7.7) (P<0.017). The PRAL<sub>shortened</sub> values were lower for 11 subjects during the Horlicks week, with a mean value of PRAL<sub>shortened</sub> for the non-Horlicks week of -2.5 mEq (SD 7.0), and a mean value for the Horlicks week of -11.5 mEq (SD 7.2) (P<0.005). Similar findings were seen for NAE<sub>Indirect</sub>.

Further work is now required on a larger and more representative sample of the UK population, but these data indicate an increase in estimates of dietary alkalinity with regular consumption of a beverage containing potassium bicarbonate

**References:**

- Catterick J., Gannon RHT., Lovell DP et al (2006). Proceedings of the Nutrition Society (in press)  
Remer T, Dimitriou & Manz F (2003) American Journal of Clinical Nutrition 77, 1255-60.

This work was funded by GlaxoSmithKline. RHTG is recipient of a University of Surrey PhD Scholarship.

## **Acid-base balance in vegetarians and non-vegetarians.**

PETER CLARYS, PETER DERIEMAEKER, DIRK AERENHOUTS,  
MARCEL HEBBELINCK, KATRIEN ALEWAETERS

Faculty of Physical Education and Physiotherapy, Vrije Universiteit Brussel, Belgium

Introduction and aims: Vegetarian diets are often considered as healthier compared to omnivorous diets. Several studies suggest that not only the diet but equally lifestyle related factors may cause the often cited better health status of vegetarians compared to non-vegetarians (Alewaeters et al., 2005). When properly matched for sex, age, BMI and physical activity level, it appeared that the nutritional intake of vegetarians was much closer to the recommendations for a healthy nutrition compared to the non-vegetarians (Deriemaeker et al., 2006).

The latter finding gave rise to more detailed analyses of some "health related" components of the daily nutritional intake of matched groups of vegetarians and non-vegetarians from our database.

It was the aim of the present study to estimate the acid-base balance in the food intake of vegetarians and non-vegetarians. It also was our aim to evaluate if additional input concerning specific food items on the existing PRAL list was necessary for the comparison of the two dietary patterns.

Methods: From our data base (300 vegetarians and 400 non-vegetarians) we selected 30 vegetarians between the age of 18 and 24 years. They were matched according to sex, age and BMI with 30 non-vegetarians. Using 3-days food records we estimated the acid-base status of the nutritional intake using the PRAL method as proposed by Remer et al (2003). Since we were working with a specific population, consuming several food items not listed in the standard PRAL table, we used additional input on acid and base balance based on the protein, phosphorus, potassium, magnesium and calcium content of the food intake derived from the Belgian Nutrient Composition Table (NUBEL). Intakes were compared using the unpaired t-test. The significance level was set at 0.05.

Results: Total PRAL values as calculated with the standard table delivered a value of  $-16 \pm 43$  mEq/d for the vegetarians compared to  $30 \pm 43$  mEq/d for the non-vegetarians ( $p < 0,001$ ). Adjusted PRAL calculation was respectively  $-33 \pm 59$  mEq/d for the vegetarians and  $38 \pm 53$  mEq/d for the non-vegetarians ( $p < 0.001$ ).

Discussion and conclusions: Our results corroborate the findings reported by Remer (2001) and indicate that vegetarian food intake brings about more alkaline outcomes compared to non-vegetarian diets. The use of the standard PRAL table was sufficient for discrimination between the two diets.

### References:

- Alewaeters K, Clarys P, Deriemaeker P, Hebbelinck M, Clarys JP. Cross-sectional analysis of body mass index and other lifestyle variables in Flemish Vegetarians. *Ergonomics* 2005; 48: 1433-44.
- Deriemaeker P, Alewaeters K, Clarys P, Hebbelinck M, Duquet W. Nutritional intake in matched vegetarians and non-vegetarians. Invited lecture Symposium "Healthy Vegetarianism", Vrije Universiteit Brussel, Brussels, Belgium, 17/12/2005
- Remer T. Influence of nutrition on acid-base balance - metabolic aspects. *Eur J Nutr* 2001; 40(5):214-20
- Remer T, Dimitriou T, Manz F. Dietary potential renal acid load and renal net acid excretion in healthy, free-living children and adolescents. *Am J Clin Nutr* 2003; 77:1255-60

## **Alkaline mineral supplementation decreased pain in rheumatoid arthritis: a randomized, controlled study.**

REGINA M. CSEUZ<sup>1</sup>, ISTVÁN BARNA<sup>2</sup>, TAMÁS BENDER<sup>3</sup>, JÜRGEN VORMANN<sup>4</sup>

<sup>1</sup>Revita Rheumatology Clinic, Budapest, Hungary; <sup>2</sup>Institute of Experimental Medicine, Budapest, Hungary; <sup>3</sup>Hospitaller Brothers of St. John's of God, Budapest, Hungary; <sup>4</sup>Institute for Prevention and Nutrition, Ismaning, Germany

**Objective:** The aim of the study was to investigate the efficacy of adding alkaline minerals in a supplementation form to the daily food intake (ordinary Western diet) as a means of suppressing the disease activity in rheumatoid arthritis (RA) patients, and to check whether any change occurs in the circulating beta-endorphin concentration.

**Methods:** Thirty-seven patients with well-controlled RA of at least two years duration, who were receiving stable pharmacological treatment, were invited to participate in a 12-week study. All patients were randomly allocated to a supplementary diet (SD) group or to a control group (CG). Their usual diet and medication was maintained. Nineteen patients in the SD group were given 30 g alkaline minerals supplement daily. Plasma immunoreactive endorphin (ir-EP) was measured in the study groups and also in healthy subjects (HS).

**Result:** DAS 28 (Disease Activity Score 28) decreased in the SD group at Visit 2 versus Visit 1: 4.7-5.2 ( $p<0.05$ ), and Visit 4 versus Visit 1: 4.5-5.2 ( $p<0.05$ ). There was no change in disease activity score during the trial in the control group. Urine pH values increased statistically significantly in the SD group ( $p<0.05$ ). The functions and emotional status (HAQ and RAQoL) of SD patients improved during the study. Negative ir-EP-DAS correlation was found in SD patients whereas in the CG patients a positive ir-EP-DAS correlation was observed.

**Discussion:** To the best of our knowledge, up till now the acid-base balance in the diet of RA patients has not been investigated. Alkaline supplementation might act as supportive therapy.

## Could minor cations and anions or other constituents such as fibers, contribute to the alkalinising properties of plant foods ?

CHRISTIAN DEMIGNÉ

Human Nutrition Unit, Institut National de la Recherche Agronomique,  
Centre de Clermont-Ferrand/Theix, France

Classical conceptions about the arousal of latent metabolic acidosis (LMA) emphasizes the role of SO<sub>4</sub> overproduction and excretion as a critical acidifying factor (generally resulting from excess protein intake), and of that K anions salts (chiefly citrate and malate) as alkalinizing factors. The aim of the present text is not question these elements, but to examine whether additional factors could modulate LMA emergence or provide alkalinising factors to be taken into accounts besides K malate or K citrate.

The connection between protein intake and some consequences of osteopenia are still disputed. In a study on a rat model adapted to different protein levels (13% and 26%), it was found that MLA was present with both levels of protein, provided that the mineral composition of the diet was non-alkalinising. This suggests that the anionic moiety of the diet plays a critical role in the acid-base balance and the prevention of MLA. *A contrario*, high urinary fluxes of sulfate do not systematically result in MLA, provided that proteins are accompanied by substantial amounts of K anions salts.

Organic anions of fruits or vegetables are essentially accompanied by potassium but also by Mg and Ca (both representing around 20% of K supply). These last divalent cations could also contribute to neutralize fixed acidity in kidneys or to reconstitute the bone Ca or Mg stores. Furthermore, factors liable to improve Mg and/or Ca absorption, such as fermentable carbohydrates in the large intestine, might have an indirect impact on MLA. This possibility is difficult to put in evidence for Ca on a rat model which presents a low basal calciuria, but seems to exist for Mg.

The question arises as to, besides K citrate or K malate, the actual role of other minor K organic anion salts that are either poorly absorbed in the small intestine or absorbed but poorly metabolized. In this view, it has been shown that a non absorbable but highly fermentable K salt such as K galacturonate is as effective as K citrate as alkalinising agent, whereas K tartrate was found poorly effective.

Taken together, these data suggest that various constituents of plant foods are liable to exert some influence on the acid-base status such as Mg organic salts and various K/Mg salts of poorly absorbed anions. The possible impact of fibers and resulting SCFA generation and absorption in the large intestine is still uncertain but awards further evaluation.

## Dietary NaCl induces low-grade hyperchloremic metabolic acidosis in healthy humans.

LYNDA A. FRASSETTO, R. CURTIS MORRIS, JR., ANTHONY SEBASTIAN  
General Clinical Research Centre, University of California, San Francisco, USA

Background: We previously demonstrated that typical American net acid-producing diets induce a low-grade metabolic acidosis of severity proportional to the diet net acid load as indexed by the steady-state renal net acid excretion rate (RNAE). We now investigate whether a sodium (Na) chloride (Cl) containing diet likewise induces a low-grade metabolic acidosis of severity proportional to the sodium chloride content of the diet as indexed by the steady-state Na and Cl excretion rates.

Methods: In the steady-state pre-intervention periods of our previously reported studies comprising 77 healthy subjects, we averaged in each subject 3-6 values of blood hydrogen ion concentration ( $[H]_b$ ), plasma bicarbonate concentration ( $[HCO_3]_p$ ), the partial pressure of carbon dioxide ( $PCO_2$ ), the excretion rates of Na ( $UNaV$ ), Cl ( $UCIV$ ), RNAE, and renal function as measured by creatinine clearance ( $CrCl$ ), and performed multivariate analyses.

Results: Dietary chloride strongly correlated positively with dietary sodium ( $p < 0.001$ ), and was an independent predictor of blood hydrogen ion concentration and plasma bicarbonate concentration, after adjustment for dietary net acid load, blood  $PCO_2$  and renal function, as demonstrated in the table below:

	UCIV	RNAE	CRCL	Blood $PCO_2$	$\beta$ -RNAE/ $\beta$ -UCIV	Intercept	$R^2$	p
<b><math>[HCO_3]_p</math></b>								
<b>b</b>	-0.012	-0.025						
<b><math>\beta</math></b>	-0.272	-0.459			1.7	+27.6	0.37	<0.001
<b>p</b>	<b>0.007</b>	<0.001						
<b>b</b>	-0.009	-0.011		+0.460				
<b><math>\beta</math></b>	-0.202	-0.203		+0.627	1.0	+7.8	0.68	<0.001
<b>p</b>	<b>0.006</b>	0.01		<0.001				
<b>b</b>	-0.010	-0.007	+0.024	+0.411				
<b><math>\beta</math></b>	-0.213	-0.141	+0.405	+0.718	0.7	+7.4	0.56	<0.001
<b>p</b>	<b>0.03</b>	0.17	<0.001	<0.001				
<b><math>[H]_b</math></b>								
<b>b</b>	+0.009	+0.021						
<b><math>\beta</math></b>	+0.224	+0.404			1.8	+37.2	0.28	<0.001
<b>p</b>	<b>0.037</b>	<0.001						
<b>b</b>	+0.011	+0.028		+0.251				
<b><math>\beta</math></b>	+0.265	+0.554		+0.368	2.1	+26.5	0.38	<0.001
<b>p</b>	<b>0.009</b>	<0.001		<0.001				
<b>b</b>	+0.010	+0.023	-0.027	+0.312				
<b><math>\beta</math></b>	+0.195	+0.399	-0.404	+0.484	2.0	+27.1	0.41	<0.001
<b>p</b>	<b>0.089</b>	0.001	0.002	<0.001				

**b**=nonstandardized regression coefficient;  **$\beta$** =standardized regression coefficient.

Conclusion: These data provide the first evidence that, in healthy humans, the diet loads of NaCl and net acid independently predict systemic acid-base status, with increasing degrees of low-grade hyperchloremic metabolic acidosis as the loads increase. Over their respective ranges of variation, NaCl has approximately 50-100% of the acidosis-producing effect of the diet net acid load (see column 6;  $\beta$ -RNAE/  $\beta$ -UCIV).

### **Bone loss because of high sodium intake: is there a connection to the acid-base balance?**

PETRA FRINGS, NATALIE BAECKER, ANDREA BOESE, MARTINA HEER  
German Aerospace Centre (DLR), Institute of Aerospace Medicine, Cologne, Germany

High dietary sodium intake is considered as a risk factor for osteoporosis. We examined the effect of increased dietary sodium on bone metabolism and acid-base balance in nine healthy male test subjects (mean age:  $26 \pm 1$  years; body weight:  $71.5 \pm 1.3$  kg). They received an individually tailored, exactly controlled nutrient intake. Only sodium intake was altered by variations in the  $\text{Na}^+$ -amount of the diet. During the first 6-day period they received a low  $\text{Na}^+$  intake, 50 mmol  $\text{Na}^+$ /d (phase 1), during the following 6-day period 200 mmol  $\text{Na}^+$ /d (phase 2). The next 10-day period they received 550 mmol  $\text{Na}^+$ /d (phase 3) and in the following 6-day period we reduced the sodium intake to 50 mmol  $\text{Na}^+$ /d (phase 4) again. The bone resorption markers (C- and N- terminal telopeptide of type I collagen (CTX, NTX)) were measured in all 24-hour urine collections. The fasting morning blood was analyzed for the bone formation markers, bone specific alkaline phosphatase (bAP) and N-terminal propeptide of type I prokollagen (PINP). Parameters of the acid-base balance (pH, bicarbonate ( $\text{HCO}_3^-$ ) and base excess (BE)) were analyzed in the capillary blood from the fingertip two times in each study phase.

NTX increased significantly from phase 1 to 2 (NTX:  $p=0.04$ ), both of the bone resorption markers increased from phase 1 to 3 (CTX:  $p<0.001$ , NTX:  $p=0.005$ ) and from phase 2 to 3 (CTX:  $p=0.01$ ; NTX:  $p=0.004$ ). The bone formation markers bAP and PINP remained unchanged in these study phases (bAP:  $p=0.47$ , PINP:  $p=0.84$ ). Between low and very high sodium intake there was a significant fall in serum pH level ( $p=0.04$ ).  $\text{HCO}_3^-$  and BE supported these changes by showing a significant fall from phase 1 to 3 (both:  $p<0.001$ ) and also from phase 2 to 3 ( $\text{HCO}_3^-$ :  $p=0.003$ , BE:  $p=0.02$ ). Nearly all bone resorption markers and acid-base parameters reached their baseline level in phase 4. We conclude that high sodium intake induces a low grade metabolic acidosis and thereby causes bone resorption.

**Estimation of the dietary acid generating potential of the elderly British population: analysis of the National Diet and Nutrition Survey (NDNS) adults aged 65 years and over using estimates of net acid excretion indirect (NAE<sub>ind</sub>) and net rate of endogenous non-carbonic acid production (NEAP).**

RICHARD H.T. GANNON<sup>1</sup>, D. JOE MILLWARD<sup>1</sup>, HELEN M. MACDONALD<sup>2</sup>, DAVID P. LOVELL<sup>1</sup>,  
LYNDA A. FRASSETTO<sup>3</sup>, THOMAS REMER<sup>4</sup>, SUSAN A. LANHAM-NEW<sup>1</sup>

<sup>1</sup>Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, United Kingdom; <sup>2</sup>Department of Medicine and Therapeutics, University of Aberdeen, United Kingdom; <sup>3</sup>Department of Medicine and General Clinical Research Center, University of California, San Francisco, United States; <sup>4</sup>Department of Nutrition and Health, Research Institute of Child Nutrition, Dortmund, Germany

Dietary intake is known to influence acid-base balance in humans under tightly controlled research conditions, but characterisation of the net effect of food groups on acid loading in population groups is ill-defined. The aims of the present study were to; (i) quantify estimates of net acid excretion indirect (NAE<sub>ind</sub>) and net endogenous non-carbonic acid production (NEAP) in a national representative group of British elderly aged 65 years and older, categorised into different age groups and between genders; (ii) compare and characterise NAE<sub>ind</sub> and NEAP in relation to food groups likely to influence dietary acid/alkaline loading; (iii) to determine regional differences in estimates of dietary acidity/alkalinity. The National Diet and Nutrition Survey (NDNS) is an ongoing joint initiative established by the Ministry of Agriculture, Fisheries and Food (MAFF) and the Department of Health. The aim of the NDNS is to provide a comprehensive, cross-sectional representation of the dietary habits and nutritional status of the population of the UK. The NDNS dataset of the British elderly consisted of a 4-day weighed dietary record and a health and lifestyle questionnaire (Finch *et al.* 1998). NAE<sub>ind</sub> and NEAP values were calculated using  $\Sigma [(protein (SO_4^{2-}) + P + *EOA) - (Mg + K + Ca)]$  and protein:K ratio respectively (Remer *et al.* 2003; Frassetto *et al.* 1998). For the purposes of this study a total of 1687 subjects were examined.

Mean NAE<sub>ind</sub> values (47.1 (SD 10.4) and NEAP (47.0 (SD 10.7) were highly correlated ( $r=0.723$ ,  $p<0.001$ ), with both estimates being higher in men. NAE<sub>ind</sub> and NEAP values increased significantly with increasing age ( $P<0.05$ ), except in the 95–104 years group due to low numbers ( $n=32$ ). Regional differences were found for intakes of NAE<sub>ind</sub>, with higher mean intakes in Scotland/North region (48.8 (SD 10.2) compared with Central/South West region (46.7 (SD 10.4) and London/South-East region respectively (45.4 (SD 10.5) ( $P<0.01$ ). Similar results were found for NEAP. NAE<sub>ind</sub> and NEAP values were inversely associated with fruit and vegetable intakes, conversely protein, meat and fish intakes were directly associated with higher NAE<sub>ind</sub> and NEAP.

These data provide an insight into the acid-generating potential of the diet in the British elderly and suggest that their diet is rich in dietary acid precursors, increasing with age and geographical location.

\*EOA was calculated using  $41 \times \text{body surface area (m}^2\text{)}/1.73 \text{ (m}^2\text{)}$  (Remer *et al.* 2003). Units of NAE<sub>ind</sub> (mEq/d) and NEAP (g/mEq/d).

References:

- Finch S, Doyle W, Lowe C, *et al.* (1998) NDNS: people aged 65 years and over. Vol 1: Report of the Diet and Nutrition Survey London, TSO.  
Frassetto LA, Todd KM, Morris RC Jr & Sebastian A (1998) AJCN 68, 576–583.  
Remer T, Dimitriou & Manz F (2003) AJCN 77, 1255–1260.

## How important is dietary acid-base balance for bone health in different age groups?

FIONA GINTY, CELIA PRYNNE, GRACIELA MUNIZ-TERRERA, GITA MISHRA, ANN LASKEY,  
LIYA YAN, ANN PRENTICE, MARIA O'CONNELL

MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge, United Kingdom

Epidemiological studies in pre- and postmenopausal women and older men and women suggest that higher fruit and vegetable intakes have a positive effect on bone health. Mechanisms have been attributed to the high alkali salt content of fruits and vegetables, which counteracts the effects of acid generating foods, such as meat and cereals. A higher dietary acid load has been shown to increase bone resorption and urinary calcium losses in adults. In a cross-sectional study spanning adolescence, early and later adulthood, we recently showed significant positive relationships between spine size-adjusted bone mineral content and fruit intake in adolescent boys and girls and older women [1]. To determine whether the fruit and vegetable effects in these subjects was attributable to lower net acid excretion, we evaluated the relationships between bone mineral measurements and two indirect estimates of acid excretion: estimated net acid excretion ( $NAE_{ind}$ ) and net endogenous non-carbonic acid excretion (NEAP).

The study group consisted of 132 boys and 125 girls (16-18 years of age), 120 young women (23-37 years of age) and 134 older men and women (60-83 years of age). Bone mineral measurements were made of the whole body, hip and spine by dual-energy x-ray absorptiometry (DXA) in all subjects. Information on health and lifestyle and physical activity was obtained by questionnaire.  $NAE_{ind}$  and NEAP were calculated from 7-day food diaries using published formulae [2, 3].

Significant positive correlations were found between  $NAE_{ind}$  and NEAP in all subjects. In general,  $NAE_{ind}$  was more strongly correlated with dairy product intake in each of the groups, and relationships with meat, fruit and vegetable intake were less consistent. This was in contrast with NEAP, which was not correlated with dairy product intake, but consistently negatively associated with fruit and vegetable intake across all groups and positively correlated with meat intake in young women and older men. In contrast to previous findings, no significant relationships were found between either measurement of acid excretion and bone mineral measurements in any age group, suggesting that our previous findings on the positive effects of fruit intake on bone could not be solely attributed to a higher alkali intake (hence a lower acid excretion). It is possible that other components of fruit may have a positive effect on bone mineral accrual and maintenance (e.g. vitamin C and other antioxidants).

Since acid-forming foods (dairy products, meat) have potential benefits for bone health at different life stages, strategies for improving dietary acid-base balance may be best served by promoting increased consumption of fruit and vegetables, rather than decreasing potentially acid foods.

### References:

- 1 Prynne CJ, Mishra GD, O'Connell MA, Muniz G, Laskey MA, Yan L, Prentice A, Ginty F (2006). Fruit and vegetable intakes and bone mineral status: a cross sectional study in 5 age and sex cohorts. *Am J Clin Nutr* 83,1420-8.
- 2 Remer T & Manz F (1995) Potential renal acid load of foods and its influence on urine pH. *J Am Diet Assoc* 95, 791-7.
- 3 Frassetto LA, Todd KM, Morris RC, Jr. & Sebastian A (1998) Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. *Am J Clin Nutr* 68, 576-583.

Funded by the UK Food Standards Agency.

## The association of dietary acidity with bone mineral density in postmenopausal women.

ANTONIA C. HARDCASTLE, DAVID M. REID, HELEN M. MACDONALD  
Osteoporosis Research Unit, University of Aberdeen, United Kingdom

Acid-base balance within the body may be upset by the Western style diet that contains a higher proportion of acid-forming animal foods compared to alkaline-forming fruit and vegetables. Increased net endogenous acid production (NEAP) together with insufficient excretion of acid by the kidneys, leads to homeostatic systems being used to buffer the excess acid. It is suggested that the dissolution of bone to release alkaline salts leads to a progressive decline in bone mineral content, which may be a contributory factor for osteoporosis. This study investigated whether dietary acidity was associated with poorer bone health in postmenopausal women.

The subjects were a subset of women who had been recruited in 1990-3 for the Aberdeen Prospective Osteoporosis Screening Study and who were invited to take part in another study in 2003 (n=289). The mean age (SD) of the women was 59.6 (2.2); they were more than 5 years postmenopausal; and were not taking hormone replacement therapy or any other treatment for their bones. They completed a four-day food diary and their bone mineral density (BMD) was measured by DXA (Lunar Prodigy) at the lumbar spine (LS) and total hip. Dietary intake was analysed using WinDiets and NEAP was calculated using the equation:  $NEAP = \{(62 \times \text{protein/mEq potassium}) - 17.9\}$  in which protein and potassium are standardised to 8 MJ, the mean energy intake of the diet <sup>(1)</sup>. The ratio of energy intake to basal metabolic rate was found to be below 1.1 for 20.4% of subjects, suggesting under-reporting or dietary restriction <sup>(2)</sup>.

NEAP was divided into quartiles (Q) and a trend was seen at LS for a lower BMD at Q4 compared to Q1 (mean (SD) 1.18 (0.17) g/cm<sup>2</sup> compared to 1.14 (0.15) g/cm<sup>2</sup>). This was significant after adjustment for age, weight, height, smoking, deprivation category and physical activity level (p=0.016). For the hip, BMD was lower for Q4 compared to Q2 and Q3 but BMD was also low at Q1 which may reflect a diet low in protein. These trends were not significant before or after adjustment for confounders.

Previous studies involving large populations have shown an association between NEAP calculated from food frequency questionnaires and markers of bone health <sup>(3,4)</sup>. Here we show an association for lower LS BMD with higher estimates of dietary acidity in a smaller study using food diaries. With regard to the hip, the lack of association may be due to limited statistical power. It has been suggested that although increased dietary acidity is associated with increased hip fracture risk, too little protein is also detrimental <sup>(5)</sup>. A balance between protein and dietary acidity may be required for optimal bone health.

### References:

- 1 Frassetto LA, Todd KM, Morris RC Jr, Sebastian A. Am J Clin Nutr 1998;68:576-83.
- 2 Goldberg GR, Black AE, Jebb SA, et al. Eur J Clin Nutr 1991;45:569-81.
- 3 New SA, Bolton-Smith C, Grubb DA, Reid DM Am J Clin Nutr 1997;65:1831-9.
- 4 Macdonald HM, New SA, Fraser WD, Campbell MK, Reid DM. Am J Clin Nutr 2005;81:923-33.
- 5 Barzel Us, Aragaki A, Ritenbaugh C et al J Bone Miner Res 2004; 19 suppl 1: s160 (abstract).

This work was funded by the Food Standards Agency. Any views expressed are the authors' own.

## **Osmotically inactive sodium retention is correlated with low-grade metabolic acidosis.**

MARTINA HEER

German Aerospace Centre (DLR), Institute of Aerospace Medicine, Cologne, Germany

Water retention has been traditionally viewed as the only physiological mechanism available to keeping serum sodium levels constant following high salt. This seems to be mandatory in order to compensate for increased postprandial serum sodium concentration. However, we have recently shown in a human metabolic balance study that high salt intake led to sodium retention without concurrent fluid retention (Heer et al. *AJP* 2000) when – starting from an already high although average sodium intake level – sodium intake is further increased. This effect called osmotically inactive sodium retention was not only shown in humans but also in rat experiments (Titze et al. *AJP* 2004). Now, the question is, where and by which mechanism can sodium be stored in an osmotically inactive way. Titze et al. (*AJP* 2004) found in their animal experiments that osmotically inactive sodium retention is paralleled by an increased mRNA expression of glycosaminoglycans in skin. If this also holds true for humans the mechanism of osmotically inactive sodium retention could be as follows. The basic assumption is that osmotically inactive sodium must be bound somewhere so that the serum sodium concentration is no more increased. A high sodium intake would induce – under determined circumstances – an increased mRNA expression of glycosaminoglycans leading to a rise of glycosaminoglycan content in the interstitial space. Glycosaminoglycans have sulphated sugar residues which could bind sodium by releasing hydrogen. If hydrogen was released, hydrogen concentration would increase in the interstitial space and possibly in blood. Concomitantly, blood bicarbonate levels as well as base excess concentration in blood would decrease. Now, this is exactly what happened in our metabolic balance study when we increased dietary sodium intake. We examined nine healthy male test subjects (age:  $25.7 \pm 3.1$  year, body weight:  $71.5 \pm 4.0$  kg, body mass index (BMI):  $21.9 \pm 3.1$  kg\*m<sup>-2</sup>) for 28 days in our clinical research facility. The study consisted of six days of 50 mEq/d Na<sup>+</sup>, six days of 200 mEq/d Na<sup>+</sup>, ten days of 550 mEq/d Na<sup>+</sup> and again six days of 50 mEq/d Na<sup>+</sup>. Fluid intake was kept constant at 40 ml/kg body weight/d. With a very high dietary sodium content mRNA expression of glycosaminoglycans increased significantly ( $p < 0.05$ ) while blood pH, bicarbonate and base excess levels decreased significantly ( $p < 0.01$ ) indicating a low-grade metabolic acidosis. Lowering sodium intake reversed the increased blood pH, and the decreased bicarbonate and base excess levels to normal.

We concluded from these results that low-grade metabolic acidosis represents a pathophysiological mechanism involved in the development of diseases associated with high sodium intake.

## **Food composition and acid-base balance: alimentary acid load and clinical implications in neonates.**

HERMANN KALHOFF<sup>1</sup>, PETER KIWULL<sup>2</sup>, FRIEDRICH MANZ<sup>3</sup>, HEIDRUN KIWULL-SCHÖNE<sup>2</sup>

<sup>1</sup>Pediatric Clinic, Dortmund; Germany; <sup>2</sup>Department of Physiology, Faculty of Medicine, Ruhr-University, Bochum, Germany; <sup>3</sup>Research Institute of Child Nutrition, Dortmund, Germany

In preterm neonates, functional limits of e.g. pulmonary and/or renal regulation processes and the considerable acid load of common formulas predispose to a great risk for the development of latent metabolic acidosis, characterized by e.g. impaired mineralization and reduced growth. To demonstrate the implications of latent acidosis in neonates, we present first results of a prospective clinical study on the development of nephrocalcinosis, second an analysis of acid-base regulation in preterm neonates under different diets, and third an algorithm to estimate the renal net acid excretion of formulas for preterm infants.

In a prospective study, very low birth weight (VLBW) infants developing nephrocalcinosis frequently showed a tendency towards metabolic acidosis (systemic pH<7.25) on day 2-7, followed by low serum levels of phosphorus (P) and high renal calcium (Ca) and P excretion within 2 weeks. Thus, impaired acid-base homeostasis with metabolic acidosis may be a further risk factor predisposing to the development of nephrocalcinosis in preterm infants.

Moreover, to obtain fundamental data of acid-base regulation in preterm infants under different diets, we investigated 48 preterm infants fed their own mother`s milk (28 native human milk, 20 enriched with fortifier) and 34 patients on formula (23 on a standard batch, 11 on a modified batch with reduced acid load). We found no notable differences between individual data of acid-base status in blood samples, irrespective of the diet. In contrast, dietary acid-base intake was accurately reflected in the urine, pointing to effective individual compensation of alimentary acid-load. Interestingly, net acid excretion (NAE) in preterm infants on human milk fortified with protein and electrolytes was only slightly higher than on native human milk in the presence of a higher urine-pH, but low when compared to standard formula.

Based on own studies and on literature data a physiologically based and empirically adjusted calculation model is presented to estimate the impact of mineral and protein content of a formula on the urinary ionogram and thus on the average renal NAE in a regularly fed and growing preterm infant.

Preterm infants fed formulas are at a considerable risk of spontaneously developing latent, and occasionally manifest, late metabolic acidosis. Renal mechanisms are predominant and effective in compensating for minor differences in alimentary acid-base intake. Thus, in preterm infants, nutritional acid-base challenges can be judged earlier and more safely by urinary than by blood acid-base analysis. The algorithm of the proposed calculation model could prove to be a useful tool in the design of new formulas with adequate base supply for preterm infants.

## Food composition and acid-base balance: experimental observations on alimentary alkali depletion in herbivores.

HEIDRUN KIWULL-SCHÖNE<sup>1</sup>, PETER KIWULL<sup>1</sup>, FRIEDRICH MANZ<sup>3</sup>, HERMANN KALHOFF<sup>2</sup>

<sup>1</sup>Department of Physiology, Faculty of Medicine, Ruhr-University, Bochum, Germany; <sup>2</sup>Pediatric Clinic, Dortmund, Germany; <sup>3</sup>Research Institute of Child Nutrition, Dortmund, Germany

Alkali enriched diets are recommended for humans to diminish their normal nutritional net acid load. As typical herbivores, rabbits on the other hand have to deal with the impact of very high dietary alkali intake on control systems involved in acid-base balance. Here it is attempted to explore the role of a high versus low alkali nutritional background for the development of chronic systemic metabolic acidosis. This may be of general interest for respiratory and renal physiology, in which research fields the rabbit serves as a common animal model in vivo and in vitro.

Data were collected from healthy male adult rabbits kept in metabolism cages, to obtain the 24h urine and blood samples from the central ear artery. Different randomized groups of animals were fed rabbit chow ad libitum providing sufficient energy but variable alkali load. One subgroup was fed high-alkali standard food and additionally received NH<sub>4</sub>Cl with the drinking water for up to nine consecutive days. Another group was fed either low-alkali food alone for five days or was given additionally NH<sub>4</sub>Cl during the last two days on low-alkali diet.

In spite of a wide range of alimentary acid-base load, normal acid-base conditions were maintained in the arterial blood [1]. In contrast, the dietary alkali load was significantly reflected by renal acid-base excretion. On high-alkali chow, an alkaline urine (pHu >8.0) was excreted, typically containing a large amount of precipitated carbonate. The average fractional renal base re-absorption was thus incomplete, in line with negative net acid excretion (NAE). On low-alkali diet, the mean pHu decreased along with a strong reduction of total base excretion. Thereby, the fractional base re-absorption was nearly complete, and NAE rose from negative values to zero level. During high-alkali feeding, a relatively large amount of added NH<sub>4</sub>Cl changed NAE to the zero level as well, but systemic BE was still maintained. During low-alkali feeding, a comparably small amount of added NH<sub>4</sub>Cl exhausted renal base re-absorption completely and gave rise to NAE above zero. This was followed by a manifest systemic metabolic acidosis, indicated by distinct reduction of base excess.

It is obvious that dietary acid-base variations are more accurately reflected in the urine than in the blood. Moreover, the urinary tract of the herbivore rabbit appears to be kept without damage despite large amounts of precipitate under nutritional conditions, which would bear growing danger of nephrolithiasis in carnivore species (e.g. cats occasionally fed too alkaline food or vegetarian humans confined to bed or meeting space-lab conditions). In the herbivore rabbit, metabolic acidosis can neither be achieved by NH<sub>4</sub>Cl against the background of normal high alkali food, nor by feeding low alkali diet alone. However, challenging renal base saving and/or net acid excretion by low alkali diet is prerequisite for growing susceptibility to NH<sub>4</sub>Cl-induced chronic metabolic acidosis in this species.

### References:

- 1 Kiwull-Schöne H, Kalhoff H, Manz F, Kiwull P. Food mineral composition and acid-base balance in rabbits. Eur. J. Nutr. 44, 499-508, 2005.

## **Does the skeleton play a role in acid-base homeostasis? Current evidence: future perspectives.**

SUSAN A. LANHAM-NEW

Centre for Nutrition & Food Safety, School of Biomedical & Molecular Sciences,  
University of Surrey, United Kingdom

With the growing increase in the age of life expectancy, hip fractures are predicted to rise dramatically in the next decade and hence there is an urgent need for the implementation of public health strategies to target prevention of poor skeletal health on a population-wide basis. Nutritional strategies for optimising bone health throughout the lifecycle are extremely important since a dietary approach is more popular amongst osteoporotic sufferers than drug intervention and long-term drug treatment compliance is relatively poor. As an exogenous factor, nutrition is amenable to change and has relevant public health implications.

The role that the skeleton plays in acid-base homeostasis has been gaining increasing prominence in the literature; with theoretical considerations of the role alkaline bone mineral may play in the defence against acidosis dating as far back as the late 19<sup>th</sup> Century. Natural, pathological and experimental states of acid loading/acidosis have been associated with hypercalciuria and negative calcium balance and more recently, the detrimental effects of 'acid' from the diet on bone mineral have been demonstrated. At the cellular level, a reduction in extracellular pH has been shown to have a direct enhancement on osteoclastic activity, with the result of increased resorption pit formation in bone.

A number of observational, experimental, clinical and intervention studies, over the last decade, have suggested a positive link between fruit & vegetable consumption and the skeleton. There is also evidence to show that: (i) the Western diet is acidic; (ii) Fad diets which are increasingly used such as the 'Atkins' diet is also very acidic; (iii) a high vegetable: animal protein ratio is effective in reducing blood pressure and may have a long-term effect on fracture risk. We now urgently need data from randomised controlled trials to determine for certain whether fruit & vegetables are important to the skeleton. A three-month intervention study involving 23-76 year old men and women has shown convincingly that a diet high in fruit and vegetables (the Dietary Approaches to Stopping Hypertension; DASH)) significantly reduces bone turnover.

The positive associations found between fruit and vegetable consumption and bone may not be due to the alkali-excess but instead to some other, identified (such as vitamin K and phytoestrogens) or unidentified 'dietary' component, with the sum being more important than the parts. Vegetables, herbs and salads which are commonly consumed in the human diet have been shown to affect bone resorption in the rat by a mechanism that is not mediated by their base excess but possibly through pharmacologically active compounds.

Currently, we have more questions than we have answers. Future research should focus attention on (i) long-term intervention trials centred specifically on fruit & vegetable intake/alkali administration as the supplementation vehicle and assessing a wide range of bone health indices (including fracture risk); (ii) experimental studies (at both the cellular, animal and human level) to determine whether there are other aspects of fruit & vegetables which are beneficial to bone metabolism and under what mechanisms.

If these questions can be answered, a 'fruit & veg' approach may provide a very sensible (and natural) alternative therapy for osteoporosis treatment, which is likely to have numerous additional health-related benefits. However, our biggest challenge remains how to persuade our Western populations to increase their fruit and vegetable consumption.

Key Words: Skeletal health, acid-base balance, systemic acidosis, potential renal acid load (PRAL), vegetarianism, fruit & vegetables, bone metabolism, dietary potassium

## **Is acid-base balance important for bone health in postmenopausal women? Evidence from cohort and intervention studies in Aberdeen.**

HELEN M. MACDONALD

Osteoporosis Research Unit, University of Aberdeen, United Kingdom

The role of acid base balance in human health is well documented. Recently there has been renewed interest in how acid-base balance affects bone health with evidence mounting from cellular work, population studies and short-term intervention trials using alkaline salts of potassium. Osteoporosis-related fractures affect one in two women and one in five men over the age of 50 years in the UK; and with aging population demographics, dietary approaches to help prevent this disease are urgently required.

Initial studies carried out in Aberdeen, North East Scotland in 1996 late premenopausal women showed that nutrients associated with fruit and vegetable intake were associated with increased bone mineral density <sup>(1)</sup>. More recently we showed that dietary acidity estimated from food frequency questionnaire was associated with reduced bone resorption in over 3000 early postmenopausal women <sup>(2)</sup>.

In order to test in the long term (2 years) whether the beneficial effects of fruit and vegetables on bone health are because of the organic salts of potassium they provide that could help balance the excess acidity caused by consuming a Westernized diet, we recruited women from our well-characterised cohort to take part in an intervention study. Women were excluded from taking part if they were taking medication for their bones (bisphosphonates, hormone replacement therapy). However, women who were on thyroxine treatment were included provided their thyroid function was stable (as assessed by free T4 and TSH levels) and their dose had not changed in the past year prior to study entry. In the potassium citrate-placebo controlled double blind intervention, women were randomized to high dose potassium citrate, low dose potassium citrate or placebo. Women could also be randomized to a fourth arm involving consuming extra 3 portions of fruit and vegetables a day. This arm was blinded only to the researcher who analysed the data.

The four intervention groups were equally matched using minimisation criteria for genotype VDR and APOE, in addition to smoking and subgroup participation. Women with the rare COL1A1 'ss' genotype were excluded. It is known that this genotype is associated with increased bone loss in women not taking HRT<sup>(3)</sup>. If these rare women were confined to one or two groups it could have a misleading impact on the outcome of the intervention study.

The study analysis has not yet been finalised and the treatment groups are still unknown at the present. However for a subset of women who provided 24 hour urine samples there was a significant difference by one way ANOVA between urinary potassium at 3 months, 6 months, 12 months, 18 months and 24 months, with no difference between the groups at baseline. Results from the intervention study regarding markers of bone health will be presented at the meeting.

### References:

- New SA, Bolton-Smith C, Grubb DA, Reid DM. Am J Clin Nutr 1997; 65:1831-9.
- Macdonald HM, New SA, Fraser WD, Campbell MK, Reid DM. Am J Clin Nutr 2005; 81:923-33.
- MacDonald HM, McGuigan FA, New SA, et al. J Bone Miner Res 2001; 16 :1634-41.

This work was funded by the Food Standards Agency. Any views expressed are the authors' own.

**Ovariectomy and dietary induced metabolic acidosis both result in decreased bone quality through different mechanisms: experience with an ovine model.**

JENNIFER M. MACLEAY<sup>1</sup>, E.S. SULLIVAN<sup>1</sup>, C.L. PECHEY<sup>2</sup>, P.A. WEST<sup>3</sup>, D.L. WHEELER<sup>1</sup>, C.M. LES<sup>2</sup>,  
A.L. BOSKEY<sup>3</sup>, A.S. TURNER<sup>1</sup>

<sup>1</sup>Colorado State University; <sup>2</sup>Henry Ford Hospital; <sup>3</sup>Hospital for Special Surgery, Fort Collins,  
United States

Rapid production of osteopenia (OP) in an animal model with bone quality that resembles human OP has been elusive to researchers. Furthermore dietary induced metabolic acidosis (DIMA) has been implicated in the development of human OP. Use of DIMA in sheep may fulfill two objectives 1) provide a rapid method to induce OP that resembles the human condition and, 2) support the hypothesis that DIMA plays a role in the development of OP in humans. We have studied a group of sheep that consumed an acidotic diet for 6 months. Four groups of 6 sheep comprised normal diet (ND), acidotic diet (MA), ND + ovariectomy (OVX) and MA + OVX. Another group of sheep with OVX and MA was evaluated at 12 months.

Treatment, especially DIMA, induced a lower blood pH, increased urinary fractional excretion of Ca and P, increased serum bone alkaline phosphatase and osteocalcin, decreased PTH, and decreased vertebral bone density as measured by dual-energy X-ray absorptiometry (DEXA). With DIMA, vertebral static and dynamic histomorphometry revealed increased bone formation rate, increased mineralizing surface and bone surface of the lumbar vertebrae and decreased percent bone and increased star volume in paired iliac crest biopsies taken at 0 and at 6 mos. There was no histologic evidence of osteomalacia. In contrast, OVX decreased bone turnover resulting in decreased percent bone and increased star volume. Mechanical testing demonstrated increased cancellous fragility and FTIR decreased mineralization. Both were more exaggerated in DIMA than with OVX. Analysis of the radii suggested that the increase in compact bone remodeling associated with either OVX or DIMA represented a process limited to secondary osteons rather than a global increase in remodeling. Mechanical testing supported an increase in viscoelasticity with anisotropy and was different between OVX and DIMA. Analysis of the femur revealed cortical thinning, increased diaphyseal perimeter, decreased BMD of the entire bone but increased BMD of cortical bone.

These results indicate that in the sheep model, DIMA induces a rapid change in cortical and trabecular bone resulting in decreased bone quality that is similar to human patients with high turnover OP. Changes in bone quality secondary to OVX are slower to occur and likely occur secondary to decreased rather than increased bone turnover.

## **Associations of biomarkers of acid load and cortisol secretion with potentially bioactive free glucocorticoids in healthy lean adults: a pilot study.**

THOMAS REMER, TRIANTAFILLIA KAN, CHRISTIANE MASER-GLUTH  
Research Institute of Child Nutrition, Dortmund, Germany

**Background:** Recent evidence suggests that endogenous glucocorticoid status is related to the body's acid load.

**Aims:** To examine whether acid load might be a predictor of potentially bioactive free glucocorticoids (UFF+UFE = sum of urinary free cortisol [UFF] and urinary free cortisone [UFE]), independently of adrenocortical activity.

**Methods:** Body composition, plasma cortisol, plasma leptin, 24-h urinary excretion rates of net acid (NAE), UFF+UFE, and further major glucocorticoid metabolites were examined cross-sectionally in a pilot study in 30 healthy adults (15 females; 22-44 years old; BMI: 20-25 kg/m<sup>2</sup>). All glucocorticoids were analyzed by RIA and leptin by ELISA. Overall daily cortisol secretion (adrenocortical activity) was assessed by the sum of the 3 major urinary glucocorticoid metabolites tetrahydrocortisone + tetrahydrocortisol + 5 $\alpha$ -tetrahydrocortisol (C21) and daily acid load by NAE.

**Results:** Plasma leptin (mean $\pm$ SD, 2.8 $\pm$ 1.6 vs. 7.6 $\pm$ 4.9 ng/mL) and percent body fat (%BF, 16.8 $\pm$ 4.2 vs. 26.9 $\pm$ 4.9 %) were lower ( $P$ <0.01) and body surface (BS)-corrected C21 higher ( $P$ <0.01) in males, whereas plasma cortisol and BS-corrected UFF+UFE were statistically undistinguishable between the sexes. Both UFF+UFE and C21 correlated positively with leptin and %BF in males ( $P$ <0.05), but not in females. Multiple regression analysis explained 72% of the UFF+UFE variability by C21, leptin, and NAE (each  $P$ <0.05) in females and 31% of UFF+UFE variability by C21 ( $P$ <0.05) in males.

**Conclusion:** Our findings indicate that apart from adrenocortical activity and the body fat product leptin, the acid load could also contribute in explaining variability of potentially bioactive glucocorticoids at least in females. More specific metabolic and larger epidemiologic studies are required to substantiate these associations.

**PRAL-independent diet effects on NEAP: acid-base considerations in stone-age sweet potato eaters, modern-day sweet potato eaters, and high-protein consumers.**

THOMAS REMER

Research Institute of Child Nutrition, Dortmund, Germany

Net endogenous acid production (NEAP) comprises of two major components: the principally anthropometrically predictable organic acid anion (OA) component and the dietary predictor PRAL (potential renal acid load). While it is widely accepted that the PRAL component can be calculated from the dietary intakes of minerals and protein, there is still some uncertainty whether particular foods or rather extreme diets may affect OA excretion irrespective of anthropometrics.

To examine PRAL-independent influences on the OA component of NEAP, literature was surveyed, measurements of dietary intakes and urinary 24-h OA excretions were performed in participants of the DONALD study, and a controlled diet experiment on the effect of a single meal of 345 g sweet potatoes on acid base status, 24-h OA and mineral excretion was conducted in 8 healthy females (32 ± 13 years).

Published data indicate that phenolic and benzoic acids found in higher amounts in certain fruits like blackcurrants, cranberries, and plums may contribute relevantly to the amount of OAs excreted. The detoxification product of benzoate, i.e., hippuric acid, has been reported to be excreted in huge amounts in some Papuan tribes of New Guinea eating predominantly highland sweet potatoes. However, despite clear changes in 24-h potassium output (+24.6 mmol/d,  $P < 0.0001$ ) and NEAP (-7.6 mEq/d,  $P < 0.05$ ), only small increases of 1-2 % were seen for total OA ( $P < 0.05$ ), hippurate ( $P < 0.05$ ), oxalate ( $P < 0.01$ ), citrate ( $P < 0.1$ ) and urate ( $P < 0.2$ ) in the sweet potato diet experiment. Literature findings suggest a higher OA excretion on higher protein intakes in formula-fed compared to human milk-fed infants and in body builders. Significant positive associations were also seen between protein intake and 24-h OA excretions ( $P < 0.0001$ ) in the currently studied groups of DONALD participants.

Irrespective of the strong dietary PRAL effect on NEAP, some particular plant foods as well as marked variations in protein intake could additionally add to variation in acid base status via alterations in OA. This may have relevance in future specification of the PRAL-NEAP model.

**Lactic acid is not the source of protons in metabolic acidosis.**

ROBERT A. ROBERGS

Exercise Physiology Laboratories, The University of New Mexico, Albuquerque, USA

The main biochemical explanation of the metabolic acidosis accompanying intense exercise has been the production of lactic acid (lactic acidosis), causing a pH dependent release of protons due to the low pKa (pH=3.87) of lactic acid. This explanation was developed based on the pioneering work of A.V. Hill and Otto Meyerhoff prior to 1922, and has remained a rigid fixture within academia and scientific research of muscle metabolism and acid-base physiology through to present time. Such a persistent acceptance of a lactic acidosis has been unfortunate for many reasons. Review of the organic biochemistry of intermediary metabolism reveals that no acid intermediates are produced from glycolysis, that lactate not lactic acid is produced from the lactate dehydrogenase reaction, and that lactate production consumes not releases a proton. Clearly, the lactic acidosis concept has no basis of support from applications of organic chemistry and metabolic biochemistry. The biochemical cause of metabolic acidosis is an increased dependence on cellular ATP hydrolysis that is not met by mitochondrial respiration. I have referred to this as an increased dependence on non-mitochondrial ATP turnover. While some exercise and acid-base physiologists have argued that the distinction between an ATP vs. lactate explanation of acidosis is trivial, the field of acid-base physiology is a good example of how such a distinction is vitally important. For example, it has been routine for physiologists to estimate proton release during muscle contraction from estimates of lactate production. Such proton release (proton load) estimates have been used to estimate tissue (structural and metabolic) buffering capacities. An incorrect acceptance of a lactic acidosis therefore causes errors in the estimation of the proton load, which in turn would cause errors in estimates of buffer capacities. I propose that the proton load of muscle contraction far exceeds lactate production, and that therefore the tissue buffer capacity (structural + metabolic) is actually far higher than previously recognized and published based on the acceptance of a lactic acidosis. The presentation ends with data resulting from my attempts to re-compute muscle buffer capacities derived from pH dependent proton loads estimated from non-mitochondrial ATP turnover.

## Drinking water composition and disease – is acidity a key factor?

RAGNAR RYLANDER

Biofact Environmental Health Research Centre, Ledum, Sweden

Since the discovery of the relation between river water acidity and death in apoplexy in Japan in 1957 a number of epidemiological investigations have reported a protective effect against cardio-vascular disease by drinking water hardness and its content of magnesium and/or calcium. In studies from Sweden, dose-response relationships were found between the drinking water content of magnesium and death in heart infarction. While a similar relation is present in some studies, others have not found it. Although it is well known that magnesium deficiency causes an increased risk for arrhythmia of the heart, muscular tonus and increased blood pressure, the results from intervention studies with magnesium or calcium on clinical markers for deficiency are ambiguous.

Mineral homeostasis is controlled by acid-base conditions in the body. Previous research has demonstrated a relation between the acid load and excretion of calcium. No information is available for magnesium. In view of this a study was undertaken where a population sample (n=85) collected 24 hour urine which was analysed for magnesium, potassium, and calcium. Net endogenous acid production was determined by measuring net acid excretion (NAE).

The results demonstrated a statistically significant relationship between NAE and magnesium ( $R^2=0.27$ ,  $p<0.0001$ ) and calcium ( $R^2=0.30$ ,  $p<0.0001$ ) but not for potassium ( $R^2=0.06$ , n.s.). There is a significant relation between the content of magnesium and bicarbonate ions in the drinking waters in Sweden. It is thus a possibility that an increased acid load due to absence of bicarbonate ions in the water (on the same time low in magnesium) caused a decreased renal reabsorption of magnesium, increasing the risk for cardiovascular disease. Support for this hypothesis is found in an intervention experiment where a reduction in blood pressure was obtained by the consumption of water rich in bicarbonate as well as magnesium. Further Intervention experiments are required to assess the importance of bicarbonate in drinking water for the homeostasis of magnesium and risk for disease.

## **Acid-base dietary components, bone mineral density, and fracture risk in the Framingham Osteoporosis Study.**

KATHERINE L. TUCKER, DOUGLAS P. KIEL, MARIAN T. HANNAN

Human Nutrition Research Centre on Aging, Tufts University, Boston, United States

Diets high in acid forming components, including several amino acids in protein foods, phosphorus and chlorine; and low in base forming components, including fruit and vegetables, potassium, calcium, magnesium and vitamin C, are hypothesized to lead to lower bone mineral density (BMD) and higher fracture risk. Some studies have shown that high animal to plant protein intake ratio may be associated with fracture. Magnesium and potassium have been shown to improve calcium and bone mineral retention. In recent years there has also been accumulating evidence of the positive effect of fruits and vegetables on BMD. We have recently shown that cola intake is associated with lower BMD, perhaps due to its phosphoric acid content. In contrast to the original hypothesis, however, we and others have found that higher rather than lower protein intake is associated with BMD. The apparent anabolic effects of the protein itself, and through increased IGF-1, appear to outweigh the negative acidic effect in typical US diets. We have recently extended our analyses from BMD to incident fracture risk in the original Framingham cohort. Of the original Heart Study subjects, 976, aged 67-95 y, completed valid food frequency questionnaires at the 20<sup>th</sup> examination. During 15 years of follow-up, there were 92 incident hip fractures. Cox-proportional hazards models were used to determine associations between magnesium, potassium, fruit and vegetables, total protein, animal protein, NEAP (net rate of endogenous non-carbonic acid production), PRAL (potential renal acid load) and dietary PRAL and hip fracture. The models were adjusted for age at exam 20, sex, BMI, height, physical activity score for the elderly (PASE), smoking status, alcohol use and total energy intake. Due, perhaps, to limitations in power, none of these variables reached statistical significance in relation to fracture risk. However, comparison of the highest versus lowest quartiles of magnesium intake and of fruit and vegetable intake resulted in non-significant Hazard Ratios (HR) in the expected direction: 0.80 (0.4-1.8) and 0.70 (0.4-1.3), respectively; and vitamin C approached significance, 0.49 (0.2-1.0), but in women only. In contrast with the original hypothesis, but consistent with our findings on protein and BMD, HRs for total protein and animal protein were also in the protective direction: 0.50 (0.2-1.2) and 0.65 (0.3-1.4), respectively. Given these results, it is, perhaps, not surprising that the NEAP, PRAL and dietary PRAL also followed this pattern: 0.67 (0.4-1.2), 0.89 (0.5-1.6) and 0.90 (0.5-1.7). Further analysis is needed with larger numbers of incident fracture. However, it appears that existing measures of acid load may need rethinking in their relation to bone, due to the apparent importance of the anabolic effects of protein that work in opposition to the negative acidic contribution.

**Dietary indicators of acid base balance and bone accrual in pubertal children.**

FRANCIS A. TYLAVSKY

Department of Preventive Medicine, University of Tennessee Health Science Center,  
Memphis, United States

Net renal acid excretion (NAE) calculated from dietary intake and body surface area has been shown to be an indicator of acid base balance as determined from 24 hour urine samples in children during puberty. However, the rapid growth may obscure the effects of diet on bone accrual. The objective of this presentation is to examine the relationship between dietary estimates of acid base balance, fruit and vegetable consumption, composite of 24 hour urinary mineral excretion and bone measurements as children transition through puberty. Two culturally diverse populations, white females residing in Memphis Tennessee USA and in Jyväskylä Finland participated in the study. Food records and 24-hour urines mineral excretion (magnesium, sodium, potassium and calcium) were collected 5 times over a 2-year period. Bone area, mineral content (BMC) and areal mineral density (BMD) of the whole body were assessed using a Dual energy x-ray absorptiometry. The girls residing in Finland were taller and had a higher body weight compared to the US cohort. Finnish girls reported consuming a more alkaline diet as estimated by predicted renal acid load (PRAL) and had lower excretion of calcium, sodium, and higher excretion of potassium than those girls residing in the US. PRAL and NAE were significantly correlated with the calculated urinary mineral composite score. Cross-sectional  $r$ , PRAL per kg, NAE per kg and calculated NAE from urine samples per body weight were negatively related to bone area, BMC and BMD of the whole body at baseline and 12 months of follow-up ( $P < 0.002$ ) but there was no relationship seen with the 24 month values. A high urinary mineral composite was related to lower accretion of bone area. These data suggest that increased metabolic acid load may jeopardize bone accrual during puberty.

## Urinary pH is an indicator of dietary acid-base load in a population: results from the EPIC-Norfolk cohort study.

AILSA WELCH, SHEILA A. BINGHAM, KAY-TEE KHAW

Department of Public Health and Primary Care, University of Cambridge, UK

**Introduction:** Dietary acid-base load is thought to be important to bone health and may also be related to other conditions (1-4). Previously, dietary acid load, measured as PRAL (potential renal acid load), has been associated with pH measured in 24-hour urine collections (5). Also small-scale intervention studies found increases in blood and urine pH with increased dietary base load (6). In a previous validation study (n=363) we found significant associations between dietary PRAL, estimated from Food Frequency Questionnaires (FFQ), and urinary pH measured in casual and 24-hour urine samples (7).

**Objective and design:** We investigated the relationship between dietary PRAL (estimated using the EPIC-Norfolk FFQ) and pH measured in casual urine samples in a cross-sectional population study of 22,038 men and women, aged 39-78 years, from the Norfolk area of the UK (8, 9). Urine pH was measured using AMES multiple reagent strips. Dietary PRAL was divided into gender specific quintiles for analysis and also adjusted for age, height, weight, physical activity, smoking habit, diagnosed high blood pressure, alcohol consumption, diuretic medication and the presence of urinary protein.

**Results:** Mean PRAL intake was -4.51 mEq/d in men and -7.22 mEq/d in women. Mean urine pH was 6.0 units in both men and women. There was a difference of 0.2 of a unit of pH in both men and women between quintiles 1 and 5 of PRAL intake, which was significant both before and after adjustment for covariates (p for trend < 0.001).

**Conclusion:** Despite the physiological influences determining urine pH and the potential error associated with using a casual urine sample, dietary acid-base load was associated with a measurable difference in urine pH within this population.

### References:

- 1 New SA, MacDonald HM, Campbell et al. *AJCN* 2004;79:131-8
- 2 Alexy U, Remer T, Manz F et al. *AJCN* 2005;82:1107-14
- 3 Welch AA, Bingham SA, Reeve J, Khaw KT. *AJCN* 2006 (submitted)
- 4 McCarty MF. *Med Hypotheses* 2005;64:380-384
- 5 Michaud DS, Troiano RP, Subar AF et al. *JADA* 2003;103:1001-1007
- 6 Buclin T, Cosma M, Appenzeller M et al. *OI* 2001;12:493-9
- 7 Welch AA, Runswick S, Mulligan A et al. *Proc Nut Soc* 2005;65:80A
- 8 Welch AA, Luben R, Khaw KT et al. *J Hum Nutr Diet* 2005;18:99-116
- 9 Remer T, Manz F. *JADA*1995;95:791-7.

## ADDRESS LIST

Name	Institution	Address	Email address
<b>Alexy, Ute</b>	Research Institute of Child Nutrition (FKE)	Heinstück 11, 44225 Dortmund, Germany	alex@fke-do.de
<b>Altland, Klaus</b>	Justus-Liebig-Universität, Institut für Humangenetik	Schlangenzahl 14, 35392 Giessen, Germany	klaus.altland@humangenetik.med.uni-giessen.de
<b>Arnett, Timothy R.</b>	Department of Anatomy & Developmental Biology, University College London	Gower Street, London WC1E 6BT, United Kingdom	t.arnett@ucl.ac.uk
<b>Ball, Derek</b>	Defence Science Technology Laboratories, Human Systems, Information Management Department	DSTL Portsmouth West, Portsmouth Hill, Fareham PO17 8AD, United Kingdom	derek.ball@abdn.ac.uk
<b>Berkemeyer, Shoma</b>	Research Institute of Child Nutrition (FKE)	Heinstück 11, 44225 Dortmund, Germany	berkemeyer@fke-do.de
<b>Burckhardt, Peter</b>	Clinique Bois-Cerf/Hirslanden, FMH médecine interne et endocrinologie	Avenue d'Ouchy 31, 1006 Lausanne, Switzerland	p_burckhardt@bluewin.ch
<b>Bushinsky, David A.</b>	University of Rochester School of Medicine, Nephrology Section Strong Memorial Hospital	601 Elmwood Avenue, Box 675, Rochester, NY 14642, United States	david_bushinsky@urmc.rochester.edu
<b>Catterick, Janet</b>	Centre for Nutrition & Food Safety, School of Biomedical & Molecular Sciences, University of Surrey	Guildford, Surrey GU2 7XH, United Kingdom	j.catterick@surrey.ac.uk
<b>Clarys, Peter</b>	Vrije Universiteit Brussel, Faculty of Physical Education and Physiotherapy	Pleinlaan 2, 1050 Brussels, Belgium	pclarys@vub.be
<b>Cseuz, Regina M.</b>	Revita Rheumatology Clinic	Margit krt. 50-52, Ground floor 9, 1027 Budapest, Hungary	recseuz@mail.datanet.hu
<b>Demigné, Christian</b>	Unité Maladies Métaboliques & Micronutriments, INRA Centre de Clermont-Ferrand/Theix	63122 St-Genes-Champanelle, France	demigne@clermont.inra.fr
<b>Frassetto, Lynda A.</b>	General Clinical Research Centre, University of California	505 Parnassus Ave, rm 1202M San Francisco, CA 94143, United States	frassetto@gcrc.ucsf.edu
<b>Frings, Petra</b>	German Aerospace Centre (DLR), Institute of Aerospace Medicine	Linder Hoehe, 51147 Cologne, Germany	petra.frings@dlr.de
<b>Gannon, Richard H.T.</b>	Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey	Guildford, Surrey GU2 7XH, United Kingdom	r.gannon@surrey.ac.uk
<b>Ginty, Fiona</b>	Medical Research Centre, Human Nutrition Research, Elsie Widdowson Laboratory	Fulbourn Road, Cambridge CB1 9NL, United Kingdom	f.ginty@btinternet.com
<b>Hardcastle, Antonia C.</b>	Osteoporosis Research Unit, Health Sciences Building, University of Aberdeen	Foresterhill, Aberdeen AB25 2ZD, United Kingdom	a.hardcastle@abdn.ac.uk
<b>Heer, Martina</b>	German Aerospace Centre (DLR), Institute of Aerospace Medicine	Linder Hoehe, 51147 Cologne, Germany	martina.heer@dlr.de
<b>Kalhoff, Hermann</b>	Westfälisches Kinderzentrum, Klinikum Dortmund	Beurhausstrasse 40, 44137 Dortmund, Germany	hermann.kalhoff@klinikumdo.de
<b>Kiwull-Schöne, Heidrun</b>	Medizinische Fakultät, Institut für Physiologie, Ruhr-Universität Bochum	Universitätsstraße 150, 44780 Bochum, Germany	heidrun.kiwull-schoene@ruhr-uni-bochum.de
<b>Lanham-New, Susan A.</b>	Centre for Nutrition & Food Safety, School of Biomedical & Molecular Sciences, University of Surrey	Guildford, Surrey GU2 7XH, United Kingdom	s.lanham-new@surrey.ac.uk
<b>Macdonald, Helen M.</b>	Osteoporosis Research Unit, Health Sciences Building, University of Aberdeen	Foresterhill, Aberdeen AB25 2ZD, United Kingdom	h.macdonald@abdn.ac.uk
<b>MacLeay, Jennifer M.</b>	Diplomate American College of Veterinary Internal Medicine, Department of Clinical Sciences, Colorado State University	300 West Drake, Fort Collins, CO 80523, United States	jmacleay@colostate.edu
<b>Remer, Thomas</b>	Research Institute of Child Nutrition	Heinstück 11, 44225 Dortmund, Germany	rem@fke-do.de
<b>Roberts, Robert A.</b>	Exercise Physiology Laboratories, The University of New Mexico	Johnson Center, Room B143, Albuquerque, NM 87131-1258, United States	rroberts@unm.edu
<b>Rylander, Ragnar</b>	BioFact Environmental Health Research Center	Bjorkasvagen 21, 443 91 Lerum, Sweden	envhealth@biofact.se
<b>Tucker, Katherine L.</b>	Jean Mayer USDA Human Nutrition Research, Center of Aging at Tufts University	711 Washington Street, Boston, MA 02111, United States	katherine.tucker@tufts.edu
<b>Tylavsky, Francis A.</b>	Department of Preventive Medicine, University of Tennessee Health Science Center	Memphis, TN 38105, United States	ftylavsky@utm.edu
<b>Welch, Ailsa</b>	The University of Cambridge, Department of Public Health and Primary Care, Strangeways Research Laboratory	Wort's Causeway, Cambridge CB1 8RN, United Kingdom	ailsa.welch@phpc.cam.ac.uk

