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Measurement Accuracy of Aluminium Content in Bone

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ABSTRACT

The aluminium content in bone has been related in several ways: to the weight of wet bone, to the weight of dry bone, to the weight of bone-ash and to the calcium content of bone. We determined the accuracy and precision of measurement (using an inductively coupled mass-spectrometer) in 30 bone samples taken from one patient. The coefficient of variation of the aluminium/weight-quotient was 12.4 per cent for wet bone, 4.7 for dry bone and 5.0 for bone ash; and the coefficient of variation of the aluminium/calcium-weight-quotient was 7.5 per cent. Thus, the aluminium content in bone seems to be best related to the weight of dry bone.

INTRODUCTION

Aluminium inhibits bone mineralization (14, 24) and the epidemic of fragility fractures has been suggested to be caused by a chronic low-grade aluminium intoxication (10, 11). However, in various studies the aluminium content in bone has been related in different ways: to the weight of wet bone (5, 6, 9, 11, 13, 18, 19, 21, 22), to the weight of dry bone (1-4, 7, 12, 16, 17), to the weight of bone ash (8, 15) and to the calcium content in the bone (10, 23). We therefore determined the means and the coefficients of variation (i.e. SD/mean) of the aluminium/weight-quotient for wet bone, dry bone and bone ash, respectively, and of the aluminium/calcium-weight-quotient in bone samples from one patient to determine the accuracy of measurement of the aluminium content in bone.

MATERIAL AND METHODS

An 89-year old woman was amputated above the knee because of knee contracture and arteriosclerotic gangrene of the foot. Immediately after the amputation 30 biopsies were taken from the trabecular bone in the femoral and tibial condyles. The bone samples were put in sealed polyethylene test tubes, frozen and stored at –20 °C until analysis.

The bone samples were randomly divided into three groups: 10 bone samples were weighed while being wet and analyzed, 10 bone samples weighed and analyzed after drying in 120°C for 48 hours, and 10 after dry ashing in 550°C for 18 hours.

The bone samples were decomposed using ultra-pure nitric acid in a quartz tube, an internal standard (Indium) added, diluted with high purity water (with a resistivity of more than 18 MΩ-cm), introduced into an inductively coupled plasma mass-spectrometer (Perkin-Elmer Elan 6000) and measured for their content of aluminium; in the first group also for their content of calcium. All handling of the samples was done in a clean room.

Quality control was assessed by the use of a certified reference material (IAEA H-8 Animal bone) in every fifth sample randomly distributed in the measurement series. The limits of detection for aluminium in bone tissue (dry weight) was assessed by producing a calibration curve. For this purpose the certified reference bone (IAEA H-8) was used. To this sample 0, 5, 10, 20, 40, 80, 160, 320, 640 and finally 1280 ng of aluminium per g dry bone was added. The standard addition procedure was consequently used to establish the calibration curve making the calculation of the limit of detection possible.

RESULTS

All samples contained aluminium. The means, ranges and the coefficients of variation (CV) of the aluminium/weight-quotients (for wet bone, dry bone and bone ash) and the aluminium /calcium-weight-quotient are given in Table 1. The hypothesis that the samples were drawn from normally distributed populations could not be rejected ($p > 0.1$, Kolmogorov-Smirnov normality test).

Table 1. Aluminium content in bone

	Mean (ng/g)	*Range (ng/g)*	*CV (%)*
Al/wet weight	351	309–457	12.4
Al/dry weight	481	451–509	4.7
Al/ash weight	715	657–745	5.0
Al/Ca	2 640	2 470–3 130	7.5

The coefficient of variation of the aluminium/weight-quotient in wet bone was larger compared to dry bone ($p = 0.01$, F-test) and bone-ash ($p = 0.01$, F-test), but there was no significant difference of this coefficient between dry bone and bone-ash ($p = 0.8$, F-test). The coefficient of variation of the aluminium/calcium-weight-quotient was in between the others and was not significantly different from any of these ($p \approx 0.2$, F-test).

The over-all accuracy was ≤ 8 per cent and the precision was ≤ 5 per cent as assessed by

the certified reference material. The limit of detection for aluminium in dry bone, defined as three times SD of the blank signal, was estimated from the calibration curve to 20.9 ng/g (Figure 1).

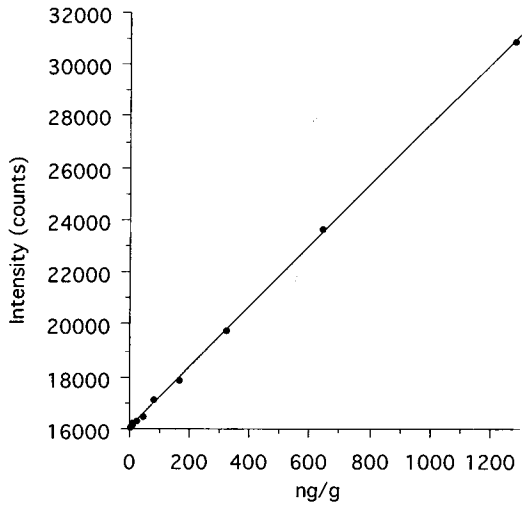

Figure 1. Digested bone calibration curve using standard addition of 0, 5, 10, 20, 40, 80, 160, 320, 640 and 1280 ng aluminium per g dry certified reference bone ($r > 0.999$, $p < 0.0001$).

DISCUSSION

The larger coefficient of variation of the aluminium/weight-quotient in wet bone compared to dry bone or bone-ash seems to be due to variable content of water in the bone samples. Although there is no significant difference between the coefficients of variation of the aluminium/weight-quotient in dry bone compared to bone-ash, the determination of aluminium is easier related to the weight of dry bone because hardly soluble aluminium compounds are formed during ashing. Also, relating aluminium to calcium does not seem to be advantageous over relating it to the weight of dry bone. Indeed, the introduction of calcium as a parameter may itself add some variation in the determination of the aluminium content in bone. Although the accuracy of the aluminium measurements may not be entirely independent of patients' bone characteristics, our result from one patient probably provides the order of the accuracy between the examined procedures.

Most investigations have used atomic absorption spectrophotometry (1-3, 5-10, 12, 13, 15-19, 21, 22); a few mass-spectrometry (4, 11). The detection limit for aluminium has been reported to be 23–150 ng/g bone tissue when determined by atomic absorption spectrophoto-

metry (8, 9, 13, 20), while it was 21 ng/g bone tissue when determined by mass-spectrometry in our study.

In conclusion, mass-spectrometry produces among the best detection limits for aluminium in bone, and the aluminium content in bone seems to be best related to the weight of dry bone.

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REFERENCES

1. Alfrey, A.C., LeGendre, G.R. & Kaehny, W.D.: The dialysis encephalopathy syndrome. Possible aluminium intoxication. N Engl J Med 294: 184-8, 1976.
2. Bouman, A.A., Platenkamp, A.J. & Posma, F.D.: Determination of aluminium in human tissues by flameless atomic absorption spectroscopy and comparison of reference values. Ann Clin Biochem 23: 97-101, 1986.
3. Boyce, B.F., Fell, G.S., Elder, H.Y., Junor, B.J., Elliot, H.L., Beastall, G., Fogelman, I. & Boyle, I.T.: Hypercalcaemic osteomalacia due to aluminium toxicity. Lancet 2: 1009-13, 1982.
4. Bush, V.J., Moyer, T.P., Batts, K.P. & Parisi, J.E.: Essential and toxic element concentrations in fresh and formalin-fixed human autopsy tissues. Clin Chem 41: 284-92, 1995.
5. Cournot-Witmer, G.C., Zingraff, J., Plachot, J.J., Escaig, F., Lefèvre, R., Boumati, P., Bourdeau, A., Garabédian, M., Galle, P., Bourdon, R., Drüeke, T. & Balsan, S.: Aluminum localization in bone from hemodialyzed patients: relationship to matrix mineralization. Kidney Int 20: 375-85, 1981.
6. D'Haese, P.C., Couttenye, M.M., Goodman, W.G., Lemoniatou, E., Digenis, P., Sotornik, I., Fagalde, A., Barsoum, R.S., Lamberts, L.V. & De Broe, M.E.: Use of the low-dose desferrioxamine test to diagnose and differentiate between patients with aluminium-related bone disease, increased risk for aluminium toxicity, or aluminium overload. Nephrol Dial Transplant 10: 1874-84, 1995.
7. Dunstan, C.R., Evans, R.A., Hills, E., Wong, S.Y.P. & Alfrey, A.C.: Effect of aluminum and parathyroid hormone on osteoblasts and bone mineralization in chronic renal failure. Calcif Tissue Int 36: 133-8, 1984.
8. Hongve, D., Johansen, S., Andruchow, E., Bjertness, E., Becher, G. & Alexander, J.: Determination of aluminium in samples from bone and liver of elderly Norwegians. J Trace Elem Med Biol 10: 6-11, 1996.
9. Leflon, P., Plaquet, R., Moriniere, P. & Fournier, A.: Determination of aluminium in bone in haemodialyzed patients, using inductively coupled argon plasma emission spectrometry. Clin Chim Acta 191: 31-8, 1990.
10. Mjöberg, B.: Aluminium-induced hip fractures. A hypothesis. J Bone Joint Surg (Br) 71: 538, 1989.
11. Mjöberg, B., Hellquist, E., Mallmin, H. & Lindh, U.: Aluminium, Alzheimer's disease and bone fragility. Acta Orthop Scand 68: 511-4, 1997.
12. O'Mahony, D., Denton, J., Templar, J., O'Hara, M., Day, J.P., Murphy, S., Walsh, J.B. & Coakley, D.: Bone aluminium content in Alzheimer's disease. Dementia 6: 69-72, 1995.

13. Radunovic, A. & Bradbury, M.W.B.: Determination of aluminium in different tissues of the rat by atomic absorption spectrometry with electrothermal atomization. Analyst 118: 533-6, 1993.

14. Rodriguez, M., Felsenfeld, A.J. & Llach, F.: Aluminum administration in the rat separately affects the osteoblast and bone mineralization. J Bone Miner Res 5: 59-67, 1990.

15. Romanski, S.A., McCarthy, J.T., Kluge, K. & Fitzpatrick, L.A.: Detection of subtle aluminum-related renal osteodystrophy. Mayo Clin Proc 68: 419-26, 1993.

16. Salusky, I.B., Coburn, J.W., Nelson, P. & Goodman, W.G.: Prospective evaluation of aluminum loading from formula in infants with uremia. J Pedriatr 116: 726-9, 1990.

17. Sedman, A.B., Klein, G.L., Merritt, R.J., Miller, N.L., Weber, K.O., Gill, W.L., Anand, H. & Alfrey, A.C.: Evidence of aluminum loading in infants receiving intravenous therapy. N Engl J Med 312: 1337-43, 1985.

18. Slanina, P., Falkeborn, Y., Frech, W. & Cedergren, A.: Aluminium concentrations in the brain and bone of rats fed citric acid, aluminium citrate or aluminium hydroxide. Food Chem Toxic 22: 391-7, 1984.

19. Slanina, P., Frech, W., Bernhardson, Å., Cedergren, A. & Mattsson, P.: Influence of dietary factors on aluminium absorption and retention in the brain and bone of rats. Acta Pharmacol Toxicol 56: 331-6, 1985.

20. Tang, S., Parsons, P.J. & Slavin, W.: Rapid and reliable method for the determination of aluminium in bone by electrothermal atomic absorption spectrometry. Analyst 121: 195-200, 1996.

21. Van de Vyver, F.L., Bekaert, A.B., D'Haese, P.C., Kellinghaus, H., Graefe, U. & De Broe, M.E.: Serum, blood, bone and liver aluminum levels in chronic renal failure. Trace Elem Med 3: 52-61, 1986.

22. Van de Vyver, F.L., Visser, W.J., D'Haese, P.C. & De Broe, M.E.: Iron overload and bone disease in chronic dialysis patients. Nephrol Dial Transplant 5: 781-7, 1990.

23. Wyatt, R.M., Ryde, S.J., Morgan, W.D., McNeil, E.A., Hainsworth, I.R. & Williams, A.J.: The development of a technique to measure bone aluminium content using neutron activation analysis. Physiol Meas 14: 327-35, 1993.

24. Zhu, J.-M., Huffer, W. & Alfrey, A.C.: Effect of aluminum on bone matrix inductive properties. Kidney Int 38: 1141-5, 1990.

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