CAPSULES notes and applications from BASi

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September 2000

Sampling Bone Minerals in Sheep Bone Muscle and Subcutaneous Interstitial Fluid with Ultrafiltration Probes

Purpose

The most abundant minerals in bone are calcium, magnesium and phosphorus. There are many circumstances in which the measurement of these minerals is important. These include nutrition studies to determine how diet affects bone health, study of bone diseases such as osteoporosis, development of drugs to combat or prevent bone diseases, and to study microgravity-induced bone loss in space and develop countermeasures to prevent it. In addition to their importance in bone, these minerals have other important functions. For example, calcium is important in muscle function, blood clotting and signaling. Magnesium is a cofactor in various enzymes, is involved in neuromuscular transmission and in ATP energy transfer. Phosphorus is ubiquitous throughout the body.

Most in vivo studies have been done by sampling blood, urine and feces. Determination of tissue concentrations usually involved post mortem harvesting of tissues. Ultrafiltration probes provide a tool for in vivo sampling of interstitial fluid in bone, muscle and subcutaneous tissue of sheep. The sheep is a good model for bone studies. It is docile and large enough to implant probes into the bone.

The interstitial concentrations of calcium, magnesium and phosphorus were determined in bone, muscle and subcutaneous tissue of normal sheep fed a normal diet.

Materials and Methods

Probes. The probes used in this study are modifications of the standard BAS Larger Animal Ultrafiltration Probe (PN MF-7028). The Bone Ultrafiltration Probe (PN MF-7029) has a reinforcing sheath to prevent kinking of the tubing as it makes a 90° bend when exiting the bone. It also has an additional tissue ingrowth cuff at the end of the sheath to promote tissue ingrowth and increase positional stability. Additional suture retainers were added to anchor the probe in place and prevent dislodging with the normal movement of the sheep. For the muscle and subcutaneous probes, only one cuff was used, but additional suture retainers were added for anchoring.

Probe implantation. The sheep were anesthetized and maintained under isoflurane general anesthesia during the implantation procedure. Strict aseptic technique was used for all surgical procedures. For bone probe implantation, a hole was drilled into the medullary cavity via the greater trochanter. A second hole was drilled into the medullary cavity at the distal portion of the shaft of the femur. A looped wire was then inserted into the proximal hole and, using a guide in the distal hole, directed out of the femur. A length of suture was then attached to the wire and withdrawn. The suture was then affixed to the probe tubing, allowing the probe to be carefully drawn into place. When the procedure was finished, the UF probe fibers were in the marrow cavity of the femur with the collection tubing exiting from the greater trochanter and then, subsequently, the skin.

For implantation into the muscle, the probe was placed into a curved introducer. Skin incisions were made at the entrance and exit points to facilitate placement. The introducer was inserted through the skin and about 7 cm into the quadriceps muscle. The curved introducer penetrated the body of the muscle and curved back to the second incision. The introducer was pulled out, leaving the probe in place. The subcutaneous probe was inserted by a similar procedure. However, a straight introducer was used since the placement was immediately under the skin. After placement of the probes, the incisions were sutured, and a suture was placed around the probes to stabilize them until the tissue ingrowth into the cuff could anchor them in place. Needle hubs were then placed on the ultrafiltration probes and the needles were inserted into Vacutainers. Fitted jackets were put on the sheep and the Vacutainers were placed into pockets in the jackets.

Sample collection and analysis. Samples were collected continuously from the UF probes. Heparinized blood samples were collected twice a week. Plasma and ultrafiltrate samples were analyzed for calcium by spectrophotometric analysis using o-Cresolphthalein Complexone method. In plasma, this analysis yields the total calcium which includes protein bound calcium, complexed calcium and ionized calcium. In the ultrafiltrate samples, this assay measures the sum of ionized and complexed calcium. In order to determine the complexed and ionized calcium in plasma, the plasma samples were ultrafiltered using U 3-2 probes. Ionized calcium was determined by ion-selective electrode. Magnesium was analyzed spectrophotometrically by the Magnon method. Phosphorus was analyzed spectrophotometrically by the formation of a phosphomolybdate complex.

Results and Discussion

The ultrafilterable calcium concentrations for the muscle, subcutaneous and bone sites were 3.50±0.09 mg/dl, 3.21±0.10 mg/dl and 2.97±0.20 mg/dl, respectively. In all tissues and in plasma, the ultrafilterable calcium was significantly higher than the ionized calcium ($p < .0001$). See ***F1***. In plasma, the total calcium (8.87±0.14 mg/dL) was significantly higher than the ionized (3.16±0.15 mg/dL) and ultrafilterable (4.35±0.15 mg/dL) ($p < 0.0001$). There were some differences in ultrafilterable calcium among the different tissues. Muscle had the highest level of ultrafilterable interstitial calcium and bone the lowest. Muscle was significantly higher than both bone ($p = 0.02$) and subcutaneous tissue ($p = 0.03$). Subcutaneous ultrafilterable calcium was not significantly higher than bone. Plasma ultrafilterable calcium was significantly higher than interstitial ultrafilterable calcium in all tissues ($p < 0.0001$). Ionized interstitial calcium concentrations in muscle, bone and subcutaneous averages were 1.77, 1.66 and 1.37 mg/dL. There were no significant differences among ionized interstitial calcium concentrations in any of the tissues. The plasma ionized calcium was significantly different from the ionized calcium in all tissues ($p = 0.0001$).

Ultrafilterable plasma magnesium (1.20±0.07 mg/dL) was significantly lower than total plasma magnesium (2.25±0.01 mg/dL), ($p < 0.0001$). The ultrafilterable magnesium concentrations for the bone, muscle and subcutaneous sites were 1.73±0.04 mg/dl, 1.58±0.04 mg/dl and 1.62±0.03 mg/dl, respectively. Bone interstitial magnesium was significantly greater than muscle and subcutaneous interstitial magnesium ($p < 0.01$). There was no difference between muscle and subcutaneous interstitial magnesium. In all tissues, the interstitial magnesium was significantly higher than the plasma ultrafilterable magnesium ($p < .01$). See ***F2***.

The mean concentrations of phosphorus in bone, muscle and subcutaneous tissue were 4.30±0.14 mg/dL, 4.35±0.14 mg/dL and 4.45±0.11 mg/dL, respectively. There were no significant differences among any of the tissues. Interstitial phosphorus in each of the tissues was significantly lower than plasma phosphorus (6.11±0.26 mg/dL), ($p < 0.0001$).

Acknowledgements

This work was funded by NASA grant NAS9-97020.

References

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2. *Janle EM, Sojka J. Use of Ultrafiltration Probes in Sheep to Collect Interstitial Fluid for Measurement of Calcium and Magnesium. Contemporary topics in Laboratory Animal Science. 39: 46-50, 2000.*

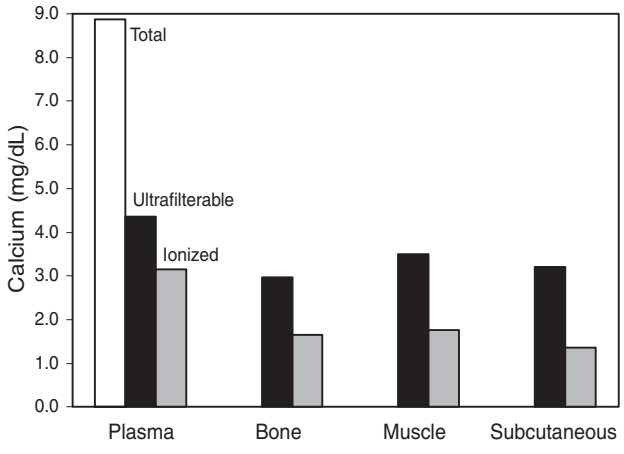

Figure 1. Calcium in plasma and interstitial tissue fluid. Ultrafilterable calcium is the ionized plus complexed calcium. Total calcium is the sum of protein bound, complexed and ionized calcium.

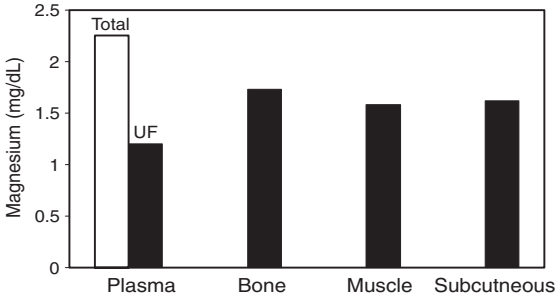

Figure 2. Magnesium in plasma and interstitial fluid.