Reprinted from Nature, Vol. 291, No. 5814, pp. 411-413, June 4 1981

Intense exercise, bone structure and blood calcium levels in vertebrates

John A. Ruben

Department of Zoology, Oregon State University, Corvallis, Oregon 97331, USA

Albert F. Bennett

School of Biological Sciences, University of California, Irvine, California 92717, USA

Sudden, large-scale fluctuations of systemic calcium concentrations are believed to be harmful to vertebrates. Consequently, it is generally accepted that vertebrates relatively closely monitor and regulate the calcium levels in blood and other tissues[1], often by endocrinological mechanisms which act over long time periods to promote calcium deposition on or dissolution from bone[2,3]. However, after intense bouts of maximal activity, virtually all vertebrates experience a significant reduction in blood *p*H as a result of concomitant increases in blood P_{CO_2} and lactate concentrations[4,5], and this might be expected to have wide-ranging physiological effects. We now present evidence that (1) blood plasma calcium concentrations rise abruptly and significantly as a result of intense muscular activity in all vertebrates investigated which have osseous skeletons, but not those with cartilaginous skeletons, and (2) the source of the excess calcium is bone.

We examined changes in plasma calcium concentrations and blood *p*H following bouts of intense activity in a variety of vertebrates of differing skeletal composition. Animals were stimulated to maximal activity until exhausted (~5 min) by chasing them by hand. Blood samples were taken from these individuals 10 min after cessation of activity. Similar determinations were also made on resting individuals. The results of these experiments (Table 1) indicate a significant rise in blood plasma calcium concentration after activity in all species with a bony skeleton ($P < 0.03$, *t*-test of each species) but no significant elevation in species with a cartilaginous skeleton (lamprey, dogfish; $P > 0.05$, *t*-test). Similar proportional increments in calcium concentration were also noted in whole blood samples taken before and after identical activity regimes in *Salmo*, *Squalus* and *Rattus*.

A separate set of experiments was conducted on the lizard *Iguana iguana* to determine the time course of activity-induced changes in blood *p*H, lactate and calcium concentrations. Results of these experiments (Fig. 1) indicate that, in *Iguana*, (1) the rise in plasma calcium concentration after initiation of activity is rapid and detectable within the first 5 min of sustainable activity, (2) the time course of elevations in plasma calcium

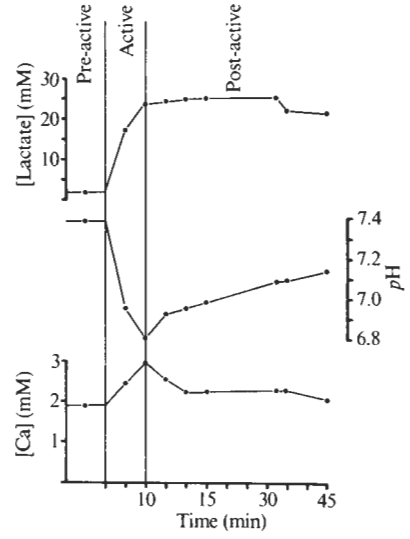

Fig. 1 Calcium and lactate concentrations and *p*H in samples of whole blood taken before, during and after 10 min of treadmill walking at 0.5 km h^{-1} in a 0.6-kg *Iguana iguana*. Three adult iguanas were exercised on a motor-driven treadmill at 35 °C. The experimental apparatus and protocol have been described elsewhere[11]. Blood was sampled before, during and after activity via an in-dwelling T-shaped cannula in the femoral artery. Anaerobically collected blood samples were analysed for *p*H as described in Table 1 legend. Additional whole blood samples were precipitated in 2× volume 0.6 M perchloric acid and analysed for [Ca] as described in Table 1 legend and [lactate] with a Boehringer–Mannheim enzymatic analysis kit[12]. Iguanas were allowed to rest for 30–60 min before pre-active blood sampling and were then exercised for 10 min at 0.5–1.0 km h^{-1}; the lizards walked steadily during the first 5 min and became fatigued and required prodding during the next 5 min. They were then allowed to recover from activity for 30–60 min. All animals experienced a rise in whole blood calcium, ranging from 0.45 to 1.15 mM, during the first 5 min of activity. Blood calcium levels peaked at 10 min activity, increasing an average of 53% above pre-active values and remained elevated for at least 30 min after exertion. Blood *p*H declined and lactate concentrations increased precipitously during activity.

Table 1 Effect of intense activity on blood *p*H and plasma calcium concentration in a variety of vertebrates (mean ± s.e.)

Species	Resting			Post-active		
	*p*H	[Ca] mM	No.	*p*H	[Ca] mM	No.
Osseous skeleton						
Rainbow trout (*Salmo gairdneri*)	7.49 ± .04	2.31 ± .01	4	7.10 ± .03	3.97 ± .16	9
Longnose gar (*Lepisosteus osseus*)	7.77 ± .04	2.56 ± .07	4	7.03 ± .05	4.14 ± .05	5
White crappie (*Pomoxis annularis*)	7.93 ± .04	2.43 ± .03	4	7.42 ± .05	2.82 ± .12	6
Largemouth bass (*Micropterus salmoides*)	7.88 ± .04	2.49 ± .06	8	7.28 ± .05	3.01 ± .05	6
Rattlesnake (*Crotalus viridis*)	7.45 ± .02	2.58 ± .08	5	6.82 ± .07	4.08 ± .13	7
Laboratory rat (*Rattus rattus*)	7.50 ± .01	2.61 ± .05	4	7.33 ± .02	3.02 ± .10	6
Human (*Homo sapiens*)	7.42 ± .01	2.65 ± .02	6	7.19 ± .02	2.88 ± .04	6
Cartilaginous skeleton						
Pacific brook lamprey (*Lampetrus pacifica*)	7.91 ± .04	2.61 ± .04	6	7.23 ± .05	2.40 ± .09	8
Pacific spiny dogfish (*Squalus suckleyi*)	7.92 ± .05	4.31 ± .07	4	7.35 ± .02	4.54 ± .11	4

To determine the resting blood *p*H, 75-μl blood samples were taken anaerobically in heparinized syringes from previously undisturbed individuals of each species. Blood was collected from the ventral aorta of fish and from tail incisions in snakes and rats. Elapsed time between first handling and sample procurement was <15 s; struggling by animals was minimal during this period. The *p*H of the sample was measured immediately with a Radiometer-Copenhagen BMS 3 mark II blood microunit connected to a Radiometer-Copenhagen PHM 71 mark II acid–base analyser. The temperature of the electrode was regulated at the body temperature of the experimental subjects. Immediately after withdrawal of the previous blood sample, another sample of ~200 μl was withdrawn from each individual in a non-heparinized syringe. Plasma from these samples was then diluted at a 1:12 ratio with a calcium-suspending solvent (containing 3.60×10^{-3} M La_2O_3; 5.0×10^{-2} M HCl) and then analysed for calcium concentration on a Jarrell–Ash atomic absorption spectrophotometer equipped with a laminar flow burner. Different individuals of each species were then stimulated to maximal activity by manual prodding until exhaustion occurred (within ~5 min). After a 10-min rest period, blood samples were collected and analysed for whole blood *p*H and plasma calcium concentration as described above. Each species was maintained and exercised at an environmentally realistic temperature (T_b = 12 °C, *Lampetra*, *Salmo* and *Squalus*; T_b = 18 °C, *Pomoxis*, *Lepisosteus* and *Micropterus*; T_b = 35 °C, *Crotalus*). Finger prick blood samples from humans were collected before and 10 min after running exercise to complete fatigue on a staircase (~4 min). Samples were analysed as above. No significant differences in blood [Ca] were noted between individuals of different sexes in any species.

concentration parallel temporal changes in blood *p*H and lactate concentration after initiation of activity, and (3) elevation of plasma calcium concentration as a result of intense activity may persist for 30 min or longer.

What is the source of this additional plasma calcium? Major reservoirs of calcium are located in bone, scales in fish, skeletal muscle and other large organs, such as the liver. To determine the role of scales in the rise of plasma calcium in osseous piscine species, plasma calcium levels before and after activity were measured in 'scaleless' trout. These animals had scales (except those associated with the lateral line system) removed by mechanical abrasion, but remained healthy. Following a 48-h recovery period, scaleless individuals were subjected to the experimentation described in Table 1 legend. Plasma calcium concentration rose by 68% following intense activity in scaleless individuals (resting: 2.42 ± 0.05 s.e. mM Ca, *p*H 7.52, $n = 5$; post-active: 3.99 ± 0.11 s.e. mM Ca, *p*H 7.17, $n = 7$) the magnitude of hypercalcaemia being almost identical ($P > 0.5$, *t*-test) to that observed after activity in normally scaled individuals (Table 1). Thus, fish scales do not seem to be the major source of calcium involved in the phenomena described here.

To investigate calcium release by soft tissues, we measured calcium concentrations of skeletal muscle and liver tissue before and after activity in rainbow trout and rattlesnakes. The results (Table 2) indicate that these tissues exhibit either static or slightly increased calcium concentrations following intense activity, rather than decreasing as would be anticipated. Thus, neither can easily be assumed to be the source of the increased plasma calcium. The lack of post-active hypercalcaemia in fish with cartilaginous skeletons implies that skeletal muscle and other soft tissues in these animals do not release significant quantities of calcium even during activity-generated acidosis. It is consequently unlikely that these tissues are the source of hypercalcemia in vertebrates with osseous skeletons. Thus, bone seems to be the most likely source of this excess calcium.

The mechanism resulting in post-active hypercalcaemia might simply involve dissolution of a fraction of the crystalline calcium hydroxyapatite compartment of the osseous skeleton as a result of systemic lactic acid accumulation and *p*H depression. This hypothesis resembles that proposed for longer-term parathormone function, in which bone resorption is thought to occur, at least in part, by local secretion of lactic acid[6]. Also, the solubility of bone calcium hydroxyapatite *in vitro* has previously been shown to be sensitive to *p*H change[7].

If increased acidity of tissue fluids associated with burst activity is indeed a causal factor in bone dissolution, our data have important implications for the evolution of the vertebrate skeleton. Recent evidence[4,5] indicates that, throughout their history, vertebrates have been capable of high levels of 'burst' activity supported by anaerobic generation of lactic acid. Activity-associated dissolution of ossified skeletal tissues resulting in marked acute hypercalcaemia may have been a problem for vertebrates since their origin and we therefore suggest that there has been continuous selection for minimization of these effects. That selection may, at least in part, account for the evolution of several seemingly unrelated skeletal features in different groups of vertebrates. For example: (1) A primarily cartilaginous adult skeleton is acquired and maintained by several distantly related groups of vertebrates, including extant cyclostomes, elasmobranchs, chimaerans and primitive actinopterygians (Chondrostei). Although cartilage is often highly mineralized, it is virtually non-vascularized, so that the relative surface areas of cartilage exposed to post-active acidic tissue fluids must be orders of magnitude less than that for bone, which is highly vascularized. Thus, far lower rates of post-active mineral dissolution and hypercalcaemia are to be expected for cartilaginous species. (2) Acellular bone develops in extinct heterostracan ostracoderms and advanced teleost fish[8]. Acellular bone in modern teleosts is distinguished from cellular bone in other vertebrates by the absence of osteocytes and osteoclasts in the former. Consequently, like cartilage, acellular bone is considerably less vascularized than cellular bone and undoubtedly has a considerably reduced surface area exposed to post-active acidic tissue fluids. (3) An epithelia-like 'barrier' of osteocytes effectively isolates the bone-tissue fluid compartment from the tissue fluid continuum of the rest of the body. Such compartmentalization, well known in mammals[9], might serve to isolate the hydroxyapatite of bone from sudden, activity-associated *p*H fluctuations experienced by the soft tissues following intense activity. Each of these various developments functions, at least in part, to isolate or 'protect' most of the calcium-laden skeletal components from body tissue fluids. They have resulted in a dramatic reduction in activity-related hypercalcaemia in animals with 'protected' skeletons compared with those with apparently little or no isolation of osseous tissues from the rest of the body (Fig. 2).

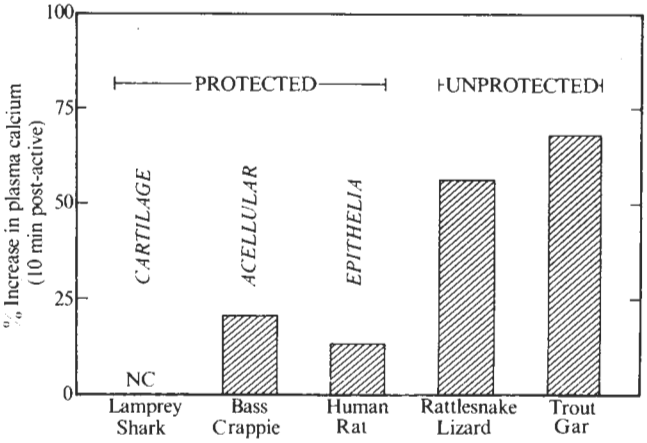

Fig. 2 Percentage increase in plasma calcium concentration after activity in vertebrates of differing skeletal type.

Thus, activity may profoundly alter blood calcium levels, even in mammals and other vertebrates with 'protected' osseous skeletons. Investigators must handle experimental subjects carefully as over-excitement may result in a rise in increments of plasma calcium concentration similar to those described here. The physiological consequences of this naturally induced, short-term hypercalcaemia have yet to be examined. However, non-activity-related acute hypercalcaemia in mammals is known to induce many deleterious physiological responses, including bradycardia, sinus arrhythmia and hypertension, disruption of normal renal function, nausea, confusion and disorientation[10]. Investigation of similar effects in vertebrates experiencing activity-related hypercalcaemia seems warranted.

This study was supported by NSF grant DEB 78-10837 (J.A.R) and grants NSF PCM 77-24208 and NIH KO4 AM00351-01 (A.F.B.). We thank T. Gleeson for assistance with

Table 2 Calcium concentration in liver and skeletal muscle before and after activity ($n = 4$ rest, 4 post-active)

	Calcium concentration (mM ± s.e.)			
	Liver		Skeletal muscle	
Species	Rest	Post-active	Rest	Post-active
Rainbow trout (*Salmo gairdneri*)	0.15 ± .02	0.18 ± .03	0.31 ± .05	0.40 ± .05
Rattlesnake (*Crotalus viridis*)	0.32 ± .03	0.33 ± .05	0.37 ± .01	0.39 ± .03

Samples of liver and skeletal muscle tissue (epaxial muscle from *Salmo* and longissimus dorsi from *Crotalus*) were obtained from decapitated specimens that had been previously undisturbed or exercised to exhaustion and allowed to recover for 10 min. Samples were homogenized in a Sorvall Omnimixer, and analysed for calcium concentration by atomic absorption spectrophotometry as described in Table 1 legend.

blood sample analysis and T. Bradley for critical evaluation of the manuscript. We also thank N. Becker, L. Case and N. Hollingsworth for assistance and the Oregon Department of Fish and Game for providing specimens of *Salmo*, *Pomoxis* and *Micropteris*.

Received 30 July 1980; accepted 20 March 1981.

1. Phang, J. M. & Weiss, 1. in *Handbook of Physiology*, Sect. 7, Vol. 7 (ed. Aurbach, G.) 157–168 (American Physiological Society, Washington DC, 1976).
2. Bentley, P. *Comparative Vertebrate Endocrinology* (Cambridge University Press, London, 1976).
3. Turner, C. & Bagnara, J. *General Endocrinology* (Saunders, Philadelphia, 1971).
4. Bennett, A. F. *A. Rev. Physiol.* **40,** 447–469 (1978).
5. Ruben, J. A. & Bennett, A. F. *Nature* **286,** 886–888 (1980).
6. Neuman, W. F., Neuman, M. W. & Meyers, C. W. *Am. J. Physiol.* **236,** 244–248 (1979).
7. McLean, F. C. & Urist, M. R. *Bone* (University of Chicago Press, 1968).
8. Hancox, N. M. *Biology of Bone* (Cambridge University Press, London, 1972).
9. Talmage, R. V. *Clin. Orthopaed.* **67,** 210–224 (1969).
10. Parfitt, A. M. & Kleerekoper, M. in *Clinical Disorders of Fluid and Electrolyte Metabolism* (eds Maxwell, M. & Kleeman, C.) 947–1151 (McGraw-Hill, San Francisco, 1980).
11. Gleeson, T. T., Mitchell, G. S. & Bennett, A. F. *Am. J. Physiol.* **239,** R174 (1980).
12. Bennett, A. F. & Licht, P. *J. comp. Physiol.* **81,** 277 (1972).