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A review of factors affecting antler composition and mechanics

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1. ABSTRACT

Antlers constitute the only mammal model for limb regeneration. A number of factors affect antler regeneration. In this review, we examine such factors and the potential consequences for organ regeneration. As body mineral stores are depleted to grow antlers, physiological exhaustion is shown in the mineral composition, mechanical performance and, according to preliminary studies, porosity of the antler bone material. Nutrition plays an important role in antler characteristics. Thus, antler composition can be used as a diagnostic tool to assess mineral deficiencies in deer. Studies on ecological effects of exceptional weather in plants suggest that minor minerals, particularly Mn, may disproportionately play roles in mechanical performance of bone material. This suggests that Mn (and perhaps other minerals) is essential to incorporate Ca and P from resorbed skeleton material into antlers. Apart from implications for game management, some effects may have applications for medicine.

2. ADVANTAGES AND CONSTRAINTS OF ANTLERS AS A MODEL FOR BONE

Other papers in this volume have addressed the suitability of antlers as a model for organ regeneration and its uniqueness in being the only mammalian organ that is regenerated each year or, indeed, at all. We will concentrate in this paper on the factors that modulate such regeneration process (i.e., factors affecting characteristics of the grown antlers). When one thinks about regeneration, as in the case of amphibian limbs or antlers, it is very likely to have the naïve idea that regenerated limbs or organs will grow as efficiently as a normal limb or organ grows during foetal development and later on during postnatal growth. I.e., that the tip of the finger in a regenerated limb must have the same size, mechanical performance of bones and their mineral composition to the fingers in pre-existing limbs. However, what we have learnt from antlers is that this is not the case, and that the physiological effort made to grow antlers affects how each section of it is grown. By physiological effort or exhaustion we indicate, in fact, an inability of the physiology to reproduce in the parts grown last the same mechanical quality, mineral composition, structure or even histological properties than in the parts grown first. Furthermore, there is a wide array of factors affecting this physiological effort that makes each section of the antler shaft along the line of growing in a different way than other sections or parts. Among them, the most important are nutrition and management (see below).

In addition to these considerations, we will also discuss how we can use all antler characteristics, from mineral composition and mechanical properties to thickness of cortical wall and histological porosity, in order to diagnose which conditions cause differences in antler quality (i.e., size, structure and mechanical performance). Antlers constitute an interesting model for research in basic bone biology because they are the only animal bone that is accessible without the interference of surgical procedures and their adverse effects.

Antlers are also relatively easy to collect in a reasonably large number of cast antlers. Many experimental procedures when studying bones imply suffering for the animals because of surgery, which involves in some cases breaking bones to assess repair. Thus, often ethical considerations recommend reducing the sample size to the minimum needed, which weakens the experiments from a statistical point of view. On the contrary, in the case of antlers, many game managers keep large numbers of them which are collected every year. As we will review below, antlers convey information regarding nutrition, environmental conditions, exceptional weather and, possibly, many more effects.

One characteristic that helps in this effort is that cast antlers take rather long to decay. Sunlight, extreme temperatures (winter frosts and summer temperatures above 35ºC which are common in Spain), and rain turn brown color of antlers into grey with visible cracks. However, antlers kept in rooms even with open windows or little isolation can keep for decades with normal aspect and color, and no visible cracks. Thus, assessing collections of cast antlers covering one or more decades in a single batch of tests allows one to learn about climate change or ecological conditions that can be hardly studied in any other animal structure. A final characteristic that facilitates such study is that antlers are related to trophy hunting, an activity historically linked to royalty or nobility, which is well known for keeping both traditions and heritage. Thus, there are many private collections of antler trophies, some of which are kept and dated as far back as the XIX century.

From the perspective of bone biology or medicine, antlers may be a good model for research as a result of most of the reasons above. In addition, antler internal structure and other characteristics are not obscured by remodeling effects. Remodeling is a process through which primary bone (i.e. the bone tissue formed during early stages of growth) is substituted by secondary bone in a process where groups of osteoclast cells 'dig' channels in the primary bone than are later lined by osteoblasts, creating new microscopic tubes of bone known as osteons (1, 2). These secondary osteons are formed at different moments throughout life and are affected by the conditions (nutrition and health conditions) when they are formed. Thus, internal bones are the result of an initial growth effort and a variable effect of secondary remodeling. As a consequence, for the purpose of assessing nutrition or other effects on internal bones, remodeling is a confounding factor that may obscure or even render important effects as non-significant in short term studies, while they may, in fact, be much more important in a long term. Antlers grow so quickly that the cortical bone tissue has hardly had time for remodeling (including our own preliminary histological studies, but see (3) for mature hard antler tissue, and (4) for remodeling in growing antler). This is further supported by the fact that the red deer antlers in our experimental farm, which are cut shortly after velvet cleaning, are as dry when they are freshly cut as those antlers kept for weeks after cutting or freshly cut antlers from game estates (all having around 15% moisture). In fact, our study showed that all antlers, regardless of origin or number of days after cutting, have an amount of moisture in dynamic balance with the surrounding air (5). Other authors, in contrast, have reported that in fallow and roe deer antlers seem to have some sort of fluid transportation into the hard antler (6-7). Thus, at least in these two species and until histological studies in antler cortical bone tissue are carried out as we have done with red deer, remodeling in mature osteons of the antler may not entirely be ruled out.

However, although it is a very interesting feature as a bone model to be free of remodeling (and thus free of a confounding effect), its particularity as a bone structure might limit in some aspects the use of antler as a bone model. The reason for this is that breaking an antler has less serious consequences for an animal than breaking an internal bone. Breaking an antler may impair fighting ability and thus end only the chances of reproduction for that season, whereas breaking a femur will lead, almost inevitably, to the death of the animal. Thus, for instance, femurs might be more highly constrained in their variability of mineral composition than antlers are. This, in turn, might affect mechanical properties, so that effects found in antlers may not be applicable to internal bones.

3. ANTLERS AS SEXUAL SECONDARY CHARACTERS

A key feature of antlers is that they are secondary sexual characters found, except for reindeer, only in males, and they are particularly costly weapons subject to a race to develop the largest size (8). They constitute 1 to 5% of body weight (7; ranging from 0.4% in spikers or one-year old males, up to 1.9% in adults under *ad libitum* diet in our farm; unpublished data by our group), and an even greater proportion of skeleton weight. Considering that the relative weight of the skeleton settles rather early at 12% of body weight (9) and considering also the data above, antlers constitute from 6.3% of the skeleton of spikers to 21% of the skeleton of adults (unpublished data by our group, and lower limit of (9); 42% according to its upper limit). This, and its fast growth rate (11-12), results in enormous growth requirements each year. The diet cannot supply minerals and protein fast enough, and thus there is a partial demineralization of the skeleton to support antler growth (13-14).

Part of the reason why antlers are so costly is that antler size is related to access to resources so that it is likely that males engage in a race to grow the largest antlers. Antler size, or even its weight, is directly related with fighting ability, access to females and place in the dominance hierarchy (15-18). The dominance hierarchy is particularly important because a higher rank position gives priority of access to food (19-21). As the most dominant males sire most of the offspring in a harem (17), there is little benefit in being second or third in the race for the largest antlers. Therefore, it is very likely that all males try to make their maximum effort to grow the largest antlers. However, only those in a good condition will succeed. Therefore, antlers can be expected to reflect male quality. Moreover, because antlers form from base or burr to top tines producing a partial demineralization of the skeleton progressively depleting stores (22), the physiological effort should be reflected in a different composition or mechanical properties along the growth axis of the antler. This, in fact, was one of the findings we have found in several studies (23-25). Another consequence of making a maximum effort to grow a structure involved in competition is that any factor affecting such effort, from male quality to nutrition, weather conditions during and just after growth, parasite load or health state, etc., should influence many of the characteristics of that structure. We will discuss such factors in detail further below. We exploited this hypothesis to use antler characteristics as a diagnostic tool to learn about male quality, nutrition level, mineral deficiencies within a deer population, management problems and even climatic influences. However, it should be noted that the yearly regeneration of an organ or structure used in sexual competition differs from what should be expected in the regeneration of another type of organ. Physiological exhaustion, influence of nutrition level, etc., should be more clearly found in organs or structures involved in sexual competition than other types of organ. If we were able to induce the regeneration of an excised hand, for example, we would not expect our body to grow the largest possible hand, but a symmetrical one. That is likely to be the case in the regeneration of limbs in some amphibians. However, the opposite case is the one for antlers. Anyway, if the organ to be regenerated is large enough (a leg or arm, for example), and because it is very likely to be above 10% of the whole body weight of an animal and probably twice or more of its skeleton weight, some effects of physiological exhaustion are also likely to be found unless the speed of regeneration is very slow.

4. MECHANICAL PROPERTIES OF ANTLERS AND INTERNAL BONES IN RELATION TO THEIR FUNCTION

If it is important to take into account that antlers are regenerated to be as large as possible, whereas a regenerating organ should be symmetrical, it is of no less importance to consider differences in mechanical function of antlers compared to internal bones. However, in order to understand these, we should first introduce the mechanical properties that we will discuss later on.

The main difference between antlers and the whole range of internal bones is that at least red deer antlers are effectively dry when they are used, whereas internal bones are soaked with water from body fluids (5, 26, but see 6-7). However, there is a wide array of functions for internal bones depending on taxon and also on the function of the organ from which the bone forms part (1, 27): from protection and lever-function, to hitting clubs or ballast. Most bones play mainly a role in protection (as in the skull) or as a stiff lever that transmits without deformation the force exerted by muscles (1, 28). The mechanical performance of whole bones, mostly their strength or force required to break them, and stiffness or resistance to deformation, is the result of: i) a combination of the overall architecture, such as cortical thickness and second moment of area (architectural resistance to bending increasing with distance of mass to the centre of gravity), and ii) bone material properties such as porosity, level of mineralization, crystal size, and properties derived from the organic phase of bone (29). As these authors point out, the resistance to fracture of a tube (e.g. long bones and antlers), depends on the thickness of the wall of the tube (or cortical thickness), but also increasing the diameter without increasing this cortical thickness (i.e. the second moment of area or architectural resistance to fracture just mentioned) can increase mechanical performance. In fact, the latter can explain 55% of variation in such performance. However, mechanical performance also depends on the quality of the material irrespective of architectural characteristics. These mechanical properties of the bone material are also called intrinsic mechanical properties, the most important being: Young's modulus of elasticity E (a measure of the material stiffness), bending strength (the maximum stress held or minimum load per unit of volume required to break a specimen), work to fracture W (which is the work necessary to break a specimen) and impact energy absorption U, which is the energy required to break a specimen in impact (1, 27, 30). We will concentrate on these intrinsic mechanical properties when we talk about mechanical properties of antlers and internal bones as calculating the mechanical performance of whole bones can be extremely complicated.

Antler material has attracted the attention of researchers in bone mechanics (5, 26, 31, 32) because, among mammalian bones, it has the highest work to fracture (27) and it is difficult to break in impact (33). In fact, a wet bone has only 15% of the impact absorption capacity (U) of dry antler, that is the state in which it is when used in fights, and the U of dry femur is only 8% that of dry antler (5, 29). Wet antler can barely be broken in impact bending, and its U is very high. In the same study, we found that antlers are nearly dry (15% moisture) in a dynamic balance with the humidity of the environment except in the days just after antler casting (22% moisture), so that we can say that antler material evolved to withstand impacts 7 times greater than those needed to break internal long bone material (5, 26). This is achieved through having the lowest mineral content in bones from 55 to 65% (23, 25, 27). As reducing the ash content reduces stiffness or *E* (1), one may think that antlers sacrificed their stiffness in order to increase *U*. However, by being dry, antlers retain 88% of the stiffness of wet femurs, and not greatly less (82%) than that of dry femur (26). Thus, most internal bones, particularly long bones, have evolved to being stiff, whereas antlers have evolved to be resistant to impact in addition to be stiff. This may be an important characteristic to take into account when considering regeneration of antlers vs. that of internal bones in limbs.

5. FACTORS AFFECTING ANTLER TRAITS: PHYSIOLOGICAL EFFORT.

Edward O. Wilson once stated that the key to understand mammals is the milk (34). In fact, lactation is the most expensive stage of reproduction (35). This is so because the risk of death is highest in most living beings in their first stages of growth. Thus, one way to reduce such risk is to shorten the risk period, which involves a high speed growth (such as should be expected in regenerating organs). Birds (except pigeons which produce a substance inadequately called milk) sustain such growth by bringing wild food to the chicks, but mammal mothers evolved milk as an adequate diet for such growth. Thus, calves can double their weight in a week at early stages of lactation (36), and milk provides not only major nutrients, but also a whole array of minerals needed for such growth (37). In a situation of high speed body and skeleton growth, minerals are so important to support it during lactation, that once major nutrients of milk, milk production, birth weight and all the important factors of lactation are included, Ca, P and Fe alone can explain 36% variability in calf growth (38). In fact, mineral supplementation during lactation has positive effects in weight and length of first antler (39).

Is the great physiological effort (i.e. metabolic activity and transfer of minerals from bones) needed to grow antlers shown in mineral composition and mechanical properties? In our first study, and sometime before we could include mechanical properties in the array of antler characteristics examined, we assessed if the effort of growing antlers affected their mineral composition. For this we used the simplest antler, the un-branched beam of spikers. In that study, we found that chemical composition of the base differed from that of the tine in terms of ash, Ca, P, K, Zn and Fe, but not Na or Mg content (23). The physiological effort was not always shown in content of minerals lower in the tip than in the base of the antler. Whereas there was less ash, Ca and P in the tip, the content of K, Zn and Fe was greater in the cortical layer. Some of these minerals were actually indices of exhaustion. K alone could explain 40% variability in weight, being greatest content in lighter antlers (23), and its effect (a greater K intake) seems to be to reduce Ca losses in urine (40). Zn is biochemically linked to alkaline phosphatase, an enzyme that starts calcium phosphate depositing in bone, so it also increases when a greater efficiency in depositing circulating Ca is needed (41). In fact, in bone that is poorly mineralized Zn is found at higher concentrations than in fully mineralized bone (42).

The change in mineral composition along the antler shaft that seems to be related to physiological exhaustion is not only found in well-fed spikers in our farm. This is also found in adult stags both in farmed and in free-ranging deer (24), and not only in minerals, but also in cortical thickness, X-ray density (43) and mechanical properties (25). Also in stags, K and Zn contents increased from base to last-grown parts of the antler (24 for this and following discussion). This study showed a U-shaped curve in ash content for farmed deer, but a continuous decrease in free-ranging deer, which suggests a greater physiological effort in the latter ones. As explained below, a similar stable pattern in farmed and decreasing content in wild antlers is shown clearly in percentages of Na and Mg along the main beam (Figure 1a and b). Could these changes play a functional role and not be an index of physiological effort? Perhaps different parts of the beam play different roles (the base of the beam having to play a greater role in absorbing impact and the top needing to be stiffer and less resistant to impact). However, it would be difficult to argue that tines at the base and those at the tip differ in their role. Thus, if differences in composition of the tines are found, they should be clearly related to physiological effort made to grow each of them. An analysis between the first, or brow tine, and the royal tines of the antlers in the farm (24) showed a thinner cortical layer, a lower content in ash and greater content of K and Mg in the top tine than in the brow tine. Fe and Zn were also greater in the top tine, but they failed to reach a statistical significance with a sample size of 15 antlers. The differences found in beam cortical thickness and Ca content were also found to affect X-ray density in farm antlers (43). Thus, these differences suggest that K, ash, cortical thickness, and possibly Zn content indicate physiological exhaustion in antler bone as a result of fast growth. It should be noted that in this paper we only examined Ca, Na, Mg, K, Zn, Fe and Si, so that similar effects may also be found in other micro-minerals.

What are the mechanical implications of these changes driven by physiological effort? A second study in the comparison between farmed and wild deer assessed intrinsic mechanical properties (25). It has already been pointed out that long bones and antlers are tubes, and, as such, their mechanical performance depends greatly on the thickness of the cortical wall. This means that the difference between the populations in cortical thickness, which depends on nutrition as we will see further below, should result in a lower mechanical performance for wild deer antler (thinner cortical wall). However there were also differences in the mechanical quality of the bone material. The stiffness, bending strength and work to fracture showed an effect of physiological exhaustion from burr to top tine which reduced their performance (Figure 2). This decrease was in general more marked for wild than farmed deer. Again, a greater amount of K, Zn, Fe, but also Si, was associated with lower stiffness (*E*) and bending strength, but not work to fracture (25). In contrast, a greater amount of Na and Mg increased these properties. It is possible that the effect is mediated through differences in porosity, but at least a rough measure of porosity was included in the analysis and controlled for. However, the examination of porosity measured at the microscopic level seems also a

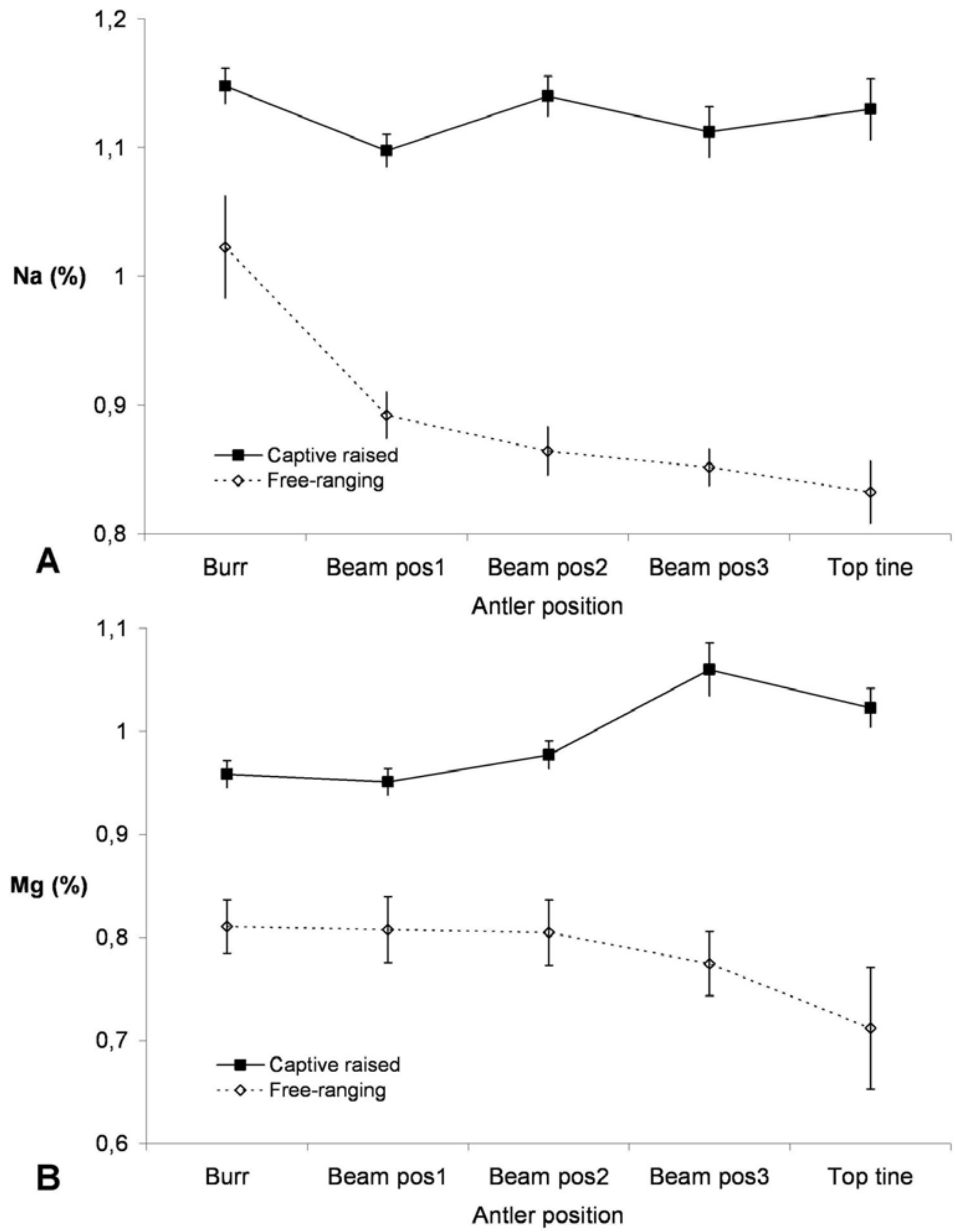

Figure 1. Trends of mineral composition in antlers of deer under high quality food (captive raised) or lower quality food. Curves differ in all sampled points with $P < 0.05$ or greater.

promising line of research: such porosity is far higher in the free-ranging deer, particularly in last parts grown, than in farmed deer (unpublished data by our group). Thus, physiological effort also seems to be linked to porosity.

In summary, a large physiological effort is linked to changes in the mineral profile, cortical thickness and porosity of the bone such that it reduces their mechanical performance. Modulating the speed of growth to reduce physiological effort, or promote mineral supply and accretion would be essential to keep symmetry of cortical thickness, bone shape, and mechanical performance in regenerated limbs compared to their counterparts.

6. FACTORS AFFECTING ANTLER TRAITS: MANAGEMENT AND NUTRITION

In general, nutrition affects various characteristics of bone. The features affected are mainly mass or density (44-47), microstructure (48) and some mechanical properties (49). However, in standard bones, such effects are often obscured by remodeling (29). This is a reason why studying effects of nutrition in antlers may constitute a

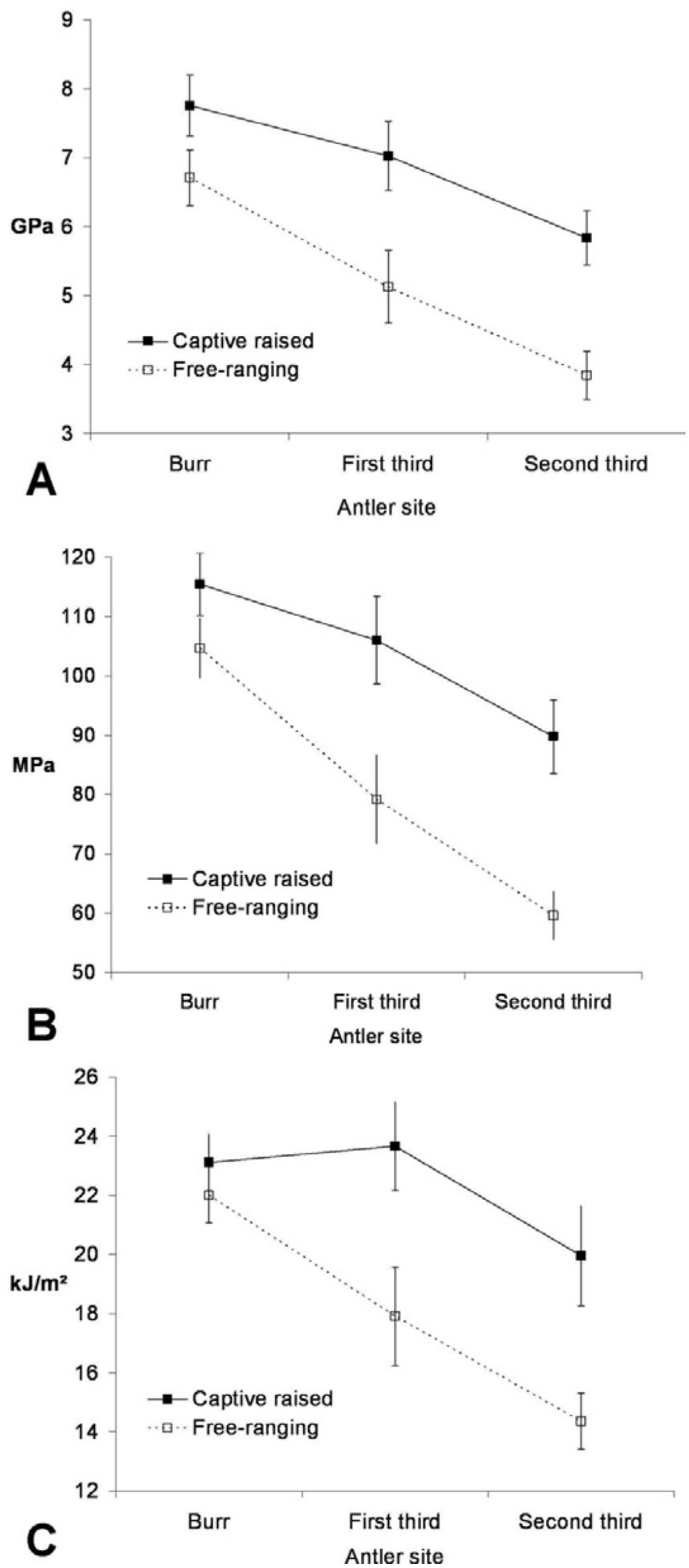

Figure 2. Trends of mechanical properties in antlers of deer under high quality food (captive raised) or lower quality food. Panel A is stiffness, B is bending strength, and C is work to fracture. Curves differ in first and second third with $P < 0.05$ or greater.

Table 1. Mineral composition of femur under supplemented diet or wild plants (control diet). Feed/vegetation ratio indicates relative mineral availability of diets

	Control	Food supplemented	P[a]	Feed/vegetation ratio
	Mean ± SE	Mean ± SE		
Ash (g/100g)	72.5 ± 0.2	72.3 ± 0.3	-	-
Ca (g/100g)	27.7 ± 0.1	27.5 ± 0.1	-	2.11
Mg (g/100g)	0.451 ± 0.003	0.447 ± 0.004	-	1.45
S (g/100g)	554.2 ± 3.9	555.4 ± 3.2	-	1.4
Na (g/100g)	0.659 ± 0.003	0.649 ± 0.004	-	18.50
P (mg/kg)	13.05 ± 0.05	13.09 ± 0.05	-	5.36
B (mg/kg)	2.40 ± 0.06	2.06 ± 0.05	0.001	0.44
Cu (mg/kg)	0.231 ± 0.006	0.250 ± 0.007	0.048	6.0
Fe (mg/kg)	1.6 ± 0.5	1.4 ± 0.8	-	3.9
K (mg/kg)	297.3 ± 2.8	282.1 ± 2.9	0.001	1.05
Mn (mg/kg)	0.26 ± 0.01	0.32 ± 0.01	0.001	5.2
Se (mg/kg)	0.42 ± 0.04	0.51 ± 0.05	-	0.46
Sr (mg/kg)	251.1 ± 3.5	238.8 ± 5.1	0.050	0.58
Zn (mg/kg)	60.0 ± 0.9	63.4 ± 1.2	0.024	14.5

[a] Dashes indicate coefficients that were not significant

.simpler model to assess more complex effects than in internal bones. In fact, the main aim of the study of the two populations of deer mentioned above was to assess if different management conditions, related to nutrition, produced changes in the mineral composition, structure and mechanical quality of the bone material. The hope behind it is that we could then use antler composition as a diagnostic tool in game management. Deer from the experimental farm were fed *ad libitum* with food containing 16% crude protein and appropriate amounts of the minerals studied (Ca, Mg, Na, K, Zn, and Fe; 49). We compared their antlers with those from a public game estate in a suboptimal habitat for deer (LD, less than 100 km from experimental farm). Food in the wild had 10% protein, and lower amounts of K, Na, Mg, and Zn, but not Ca or Fe. In fact, the largest ratios were for Na, Mg and K, where content in the diet of farm deer was 6.3, 3.1, and 2.2 times greater, respectively, than in the plants available for free-ranging deer (49). This is not likely to be unusual in wild diets: these are known to be often deficient in Na, Mg, P, and K (50-52). The examination of differences in the mineral composition of antlers between both populations matched the largest ratios: the mean composition of antlers differed in Na, Mg and K, although not in Zn. Thus, antlers reflect the differences found in the diet. The examination of trends in mineral content along the main axis of growth in the antler (24-25) show clearly in the case of Na and Mg that the content in these minerals in free ranging deer decrease sharply as the antler is finished, reflecting a probable depletion of the body stores (Figure 1a and 1b). As mentioned, this is linked to the mechanical properties, which suggest that good nutrition and body stores are particularly important during a regeneration process such as it might be the case in limb regeneration (Figure 2a, 2b and 2c).

Could it be different for internal bones? We have examined the effects of supplementary food available for hinds over 3 years (starting just after weaning). One of the groups had *ad libitum* access to supplementary feed rich in most minerals in addition to wild vegetation, whereas another group only had access to wild plants. The differences found between groups in mineral composition of the femur, except for Na and P, matched the greatest ratios between food supplement and the plants which usually constitute the diet of deer in the wild (CA Olguín, unpublished data). The results on factors affecting mineral composition of bone extend those found previously in antlers, as we examined 20 minerals in this study. Femurs differed in Mn, Cu, and Zn between supplemented and control groups, reflecting a mean content of these minerals in the diet 8.5 times higher in the food supplement compared to plants constituting the diet of deer (Table 1). It is particularly interesting that giving *ad libitum* access to food supplement to one of the groups only increased 7.2% the body weight of hinds, it increased slightly body condition, and supplementation had no effect in body height, femur length or cortical thickness. This suggests that minor but consistent (long term) changes in the diet producing small changes in body weight and no changes in body (and bone) size may nevertheless produce changes in bone mineral composition reflecting the diet in trace, but not major minerals. This, in turn, suggests that if limb regeneration was possible, diet is likely to influence the composition and mechanical properties of bones in regenerated limbs. It will be particularly interesting to assess which changes in the diet may result in the increased level of porosity that we mentioned earlier, and if this effect of diet is also exerted in internal bones.

An effect that may be considered as a particular case of nutrition is that of weather. Nutrition effects have proved to be the underlying factor of environmental effects on antler length in spikers (39, 54-55). Plant productivity has long been known to be affected by inter-annual variations in climate (56). Studies in South Spain, a country that has a dry climate, showed that overall rainfall of the year in which the antler was grown affected the quality of the antler (57). Although effects of climate in these and the study discussed below seem to be related to availability of food or its composition, it should be borne in mind that other effects of climate cannot be ruled out, such as increasing parasite abundance (58-59), insect activity (60) or the costs related to extreme wind in winter (61).

We have found that North Atlantic Oscillation (NAO), a large-scale climate index shown to be predictor of ecological processes (56, 62-63; JA Gómez, unpublished

Table 2. Antler characteristics in years greatly differing in incidence of fracture (see section 6) under a wild diet in LM game estate, or under a wholemeal diet in University farm.

Antlers from wild deer at LM game estate	SW – 2004[a]	LWF - 2005	P
Cortical thickness, mm	5.65 ± 0.28	4.65 ± 0.2	0.005
Physical density, g/cm^3	1.750 ± 0.006	1.722 ± 0.010	0.027
Mean shaft diameter, mm	4.14 ± 0.11	3.85 ± 0.06	0.023
Impact work, kJ/m^2	54.9 ± 2.7	40.1 ± 1.8	0.001
Young's modulus, GPa	15.69 ± 0.32	15.22 ± 0.39	> 0.1
Bending strength, MPa	306.6 ± 6.4	299.0 ± 7.6	> 0.1
Work to peak force, kJ/m^2	38.0 ± 1.5	34.2 ± 1.1	0.045
Ash, %	62.3 ± 0.2	61.5 ± 0.5	0.095
Ca, %	21.0 ± 0.2	20.3 ± 0.2	0.012
P, %	10.1 ± 0.1	9.8 ± 0.1	0.033
Mg, %	0.464 ± 0.007	0.462 ± 0.005	> 0.1
Na, %	0.591 ± 0.007	0.567 ± 0.004	0.004
K, mg/kg	578 ± 32	549 ± 17	> 0.1
Sr, mg/kg	213 ± 8	212 ± 6	> 0.1
Si, mg/kg	54 ± 4	100 ± 11	0.001
Mn, mg/kg	4.48 ± 0.25	3.52 ± 0.42	0.056
Cu, mg/kg	0.258 ± 0.014	0.290 ± 0.012	0.093
Fe, mg/kg	23.1 ± 1.2	29.3 ± 2.2	0.019
Zn, mg/kg	57.9 ± 1.7	55.8 ± 1.6	> 0.1
B, mg/kg	2.94 ± 0.21	2.68 ± 0.09	> 0.1
Co, mg/kg	0.224 ± 0.014	0.169 ±0.018	0.024
Antlers from deer at university farm[b]			
Antler length, cm	75 ± 2	88 ± 2	0.001
Deer weight, kg	191 ± 8	209 ± 6	0.062
Ca, %	19.0 ± 0.2	19.9 ± 0.2	0.001
P, %	9.3 ± 0.1	9.8 ± 0.1	0.004
Mg, %	0.49 ± 0.1	0.49 ± 0.1	> 0.1
Na, %	0.61 ± 0.01	0.66 ± 0.02	0.024
K, mg/kg	718 ± 36	650 ± 24	> 0.1
Sr, mg/kg	427 ± 17	407 ± 18	> 0.1
Si, mg/kg	114 ± 14	84 ± 13	> 0.1
Mn, mg/kg	0.88 ± 0.22	0.57 ± 0.07	> 0.1
Cu, mg/kg	0.25 ± 0.02	0.34 ± 0.07	> 0.1
Fe, mg/kg	29 ± 2	30 ± 7	> 0.1
Zn, mg/kg	48 ± 2	54 ± 1	0.05
B, mg/kg	3.7 ± 0.1	2.2 ± 0.1	0.001
Co, mg/kg	0.23 ± 0.01	0.28 ±0.01	0.05

[a] The table shows a sample of cast antlers (13 broken and 7 intact in LWF vs. 15 intact and 5 broken in SW). [b] For an unplanned comparison the bottom of the table shows only the chemical composition of antlers from 11 males in the experimental deer farm

data) explained a mean of 32% variability in length, weight and other important measures of 7783 cast antlers collected from 1985 to 2006 in central Spain (JA Gómez, unpublished data). We do not know if the effects of NAO on antler characteristics are exerted through effects on plant productivity (i.e., amount of food), mineral composition, or other factors such as parasite abundance, etc.

Antlers also provide an exceptional natural model for assessing various effects on bone mineral composition and mechanical properties. Some of these experiments may be pivotal to understand bone biology. One of them regards a study initiated after several game estate managers came to us indicating that, throughout Spain, the antlers grown after a short period of very intense cold in late January-early February in 2005 broke at a very high rate and often in the main beam. Usually, a small proportion of antlers break at the main beam or in several tines. A similar effect in femurs or other internal bones would be obscured in the sense that the animal would die after a few days. Thus, it may not be clear if a loss of body condition or illness reduced body weight and produced finally a bone fracture, or, on the contrary, the fracture was the cause of death. In antlers, however, one can discern effects on antler bone composition and mechanical properties from an otherwise healthy animal. Thus, we designed the study including only two years: one of exceptional cold weather in late winter (termed late winter frosts or LWF) and the standard preceding year (termed standard winter or SW; 65). LWF produced a large effect in antler breakage (55% of serious breakage of 3 or more tines, which included 33% antlers broken in the main beam vs. 25% of serious breakage the preceding year including 9% broken in the main beam). The effect also reduced antler weight, as all the antlers collected (about a hundred each year) in LWF had 75% of the weight in those of the SW year. The effect was not attributed to a reduction in food availability, as there was no difference in body weight between the animals shot for trophy and population reduction in LWF or SW.

However, the most interesting results were those regarding a subsample of antlers broken or intact within LWF and SW year. When we compared antlers from LWF and SW we found that LWF was associated with reduced impact energy (*U*) and somewhat reduced work to fracture (*W*)**,** Young's modulus (*E*), cortical thickness and physical density (Table 2). I.e., the greater incidence of fractures may be partly explained in terms of lower impact energy and work to fracture (work or energy needed to break in impact and slow bending, which achieved 27% and 10%, respectively). LWF was associated with considerably increased Si and reduced Na. To a lesser extent other minor minerals were also affected. However, one of the most interesting effects was that of Mn: this was reduced in LWF year, but the greatest difference was not between LWF and SW antlers, but a lower content in Mn in the case of broken antlers in both years compared to the intact ones. No such effect was found in farmed deer fed whole meal and enduring the same cold winter (and antler size was not reduced either), so that the effect seemed to be mediated by nutrition. The first step of this effect seemed to be an increase in Si by plants in response to stress caused by cold: the effect of incorporating Si by all sorts of crops and wild plants in response both to biotic and abiotic stresses is widely documented (reviewed by 66-68). Si is taken up by plants and counteracts attacks by fungi, insects, plant diseases, and the effects of salinity, drought, freezing, and toxic levels of Al, Cd, and Mn (68). Thus, freezing temperatures might have triggered the uptake of Si in plants in the LWF year producing, as a side effect only in the game estate and not in the farm, the reduction of Mn and Na. Mn rather than Na seemed to be the important mineral linked to a reduction in impact work and work to fracture. The evidence for this conclusion came from a comparison between well-fed farm deer and those in another game estate (65). The deer from the game estate had a food of lower quality and antlers showing deficiency in Na, Mg, Co, Cu and Mo, but they were higher than farm antlers in content of Mn. Despite the greater cortical thickness of farm antlers and, presumably, greater whole antler mechanical performance derived from structure, work to fracture was a 40% smaller in the antler material from the farm than that from the game estate (65). Thus, only lower Mn content and not that of Na was linked to bone material properties. Ca and P were unlikely to be a problem in this study and in general in those involving antlers because, as mentioned above, antlers are grown mainly using minerals from the animal own skeleton. In addition, Ca and P were unlikely to have caused changes in mechanical properties because a slight reduction in Ca and P in LWF would be expected to have increased the impact energy and work to fracture (33), in contrast to what was found.

Thus, minor changes in bone minerals induced by diet, may have marked effects in mechanical properties of bone even when there is not a shortage of the two principal minerals, Ca and P. This certainly has implications for management. At least in habitats with deficiency of Mn, even if there is no lack of Ca and P, just the addition of this mineral may increase antler weight by 30%, as well as improving mechanical performance, structural variables and bone density.

In conclusion, antlers seem to be a good model not only to study bone and organ regeneration, but also factors affecting this, such as nutrition, physiological exhaustion, mineral turnover, and others.

7. PERSPECTIVES

Antler research on variation in mineral composition and their associated changes in mechanical properties and histological structure is likely to become a valuable research trend in bone biology. Antlers are so easily accessible and at such number that we can learn of unexpected effects in their growth or mechanical performance that would be obscured in internal bones. Studying these effects, as well as traditional experiments designed by a researcher may give us insights into bone biology that may be applicable from game management to medicine.

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