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BONE DENSITY AND CALCIUM AND PHOSPHORUS CONTENT OF THE GIRAFFE (*GIRAFFA CAMELOPARDALIS*) AND AFRICAN BUFFALO (*SYNCERUS CAFFER*) SKELETONS

by

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PREFACE

The work described in this dissertation was carried out in the Veterinary Wildlife Unit, Faculty of Veterinary Science, University of Pretoria under the supervision of Professors J.D. Skinner and G. Mitchell.

Some aspects of the work reported in this dissertation have already been published/submitted for publication, but are not cited separately as their contents have been incorporated in this work. The relevant papers are:

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SUMMARY

Bone density and Calcium and Phosphorus content of the giraffe (*Giraffa camelopardalis*) and African buffalo (*Syncerus caffer*) skeletons

by

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Apart from its slender appearance, four main factors lead to questions regarding the bone density, mineral content and morphology of the giraffe skeleton:

- A rapid vertical growth rate – especially in the neck and metapodials
- Biomechanical considerations pertaining to the tall and slender shape of the skeleton
- A proportionally larger skeleton in relation to body mass
- A seemingly abnormal mineral balance in their diet with possible signs of mineral deficiency (i.e. osteophagia)

In this study the skeleton of the giraffe was compared with that of the African buffalo with regards to bone density, skeletal calcium (Ca) and phosphorus (P) content and certain femoral and metacarpal morphological characteristics. The aim was to establish if, compared to buffalo, the features of the giraffe skeleton differed in any unique way.

Fourteen similar bones or parts of bones were collected from carcasses of six adult giraffe bulls and nine adult buffalo bulls. These bones were cleaned, weighed and their volume determined through water displacement, from which their density could be calculated. Hereafter, Ca and P content were analysed in 10 bones from each carcass. Morphological characteristics of cross-sections from femoral and metacarpal shafts were also measured.

No significant differences between the density or mineral content of bones in the two species could be found. In both species 19,5% Ca and 9,5% P were measured in defatted bone. Although similar in mineral concentration, the giraffe skeleton contains three times more absolute Ca and P, which translates into a 1,5-2-fold higher dietary requirement for these minerals compared to buffaloes. A gradation in the volume and weight of cervical vertebrae was also seen in giraffes. This could hold biomechanical advantage for the carriage and manoeuvrability of the long neck. Bone wall thickness of the giraffe femur and metacarpus is increased compared to buffaloes. This could hold biomechanical advantage for the slender legs that are subjected to increased vertical forces.

Adequate Ca seems to be acquired through very specific browse selection, which seems to be of evolutionary origin, while the acquisition of adequate P seems to be critical and a possible cause for osteophagia. This study is the first of its kind in these species and therefore also provide valuable baseline data for future work in this field.

CHAPTER I

INTRODUCTION

The giraffe *Giraffa camelopardalis* is a truly fascinating animal. It is unique in both appearance and demeanour and it is these attributes that have interested both scientists and travellers for centuries. Despite this enchantment, very little research has been done on this species. What has been done, has focussed mainly on the evolution, habits, feeding ecology and some specific physiological attributes (cardiovascular, respiratory and thermoregulatory) of the giraffe. No workers have looked at the skeletal physiology of this species and only few at its skeletal biomechanics.

Four main factors lead to questions regarding the bone density, mineral composition and morphology of the giraffe skeleton:

- A rapid vertical growth rate
- Biomechanical considerations pertaining to the shape of the skeleton
- Proportion of body mass that is skeleton
- Dietary mineral balance and requirements

In this study the skeleton of the giraffe is compared with that of the African buffalo *Syncerus caffer* with regards to bone density, skeletal calcium (Ca) and phosphorus (P) content and certain femoral and metacarpal morphological characteristics, to establish if there are any unique features, in this regard, in the giraffe's skeleton.

1.1 LITERATURE REVIEW & BACKGROUND

Rapid vertical growth rate

Mature giraffe bulls grow to an average height of 4.9-5.2m (Skinner & Smithers, 1990), making them the tallest animal in the world. Their vertical growth rate is rather astounding, especially during the first year of life. For example, Dagg & Foster (1976) reported a one meter increase in height within the first six months after birth, of which up to 23cm can be added in one week. They also mention that much of this initial growth appears in the neck, as was likewise reported by Simmons & Scheepers (1996) when they compared the foreleg to neck ratio of young to older giraffes. This disproportionate growth rate of the neck, compared to the rest of the body, was already demonstrated by Slijper in 1946, when he reported that cervical vertebrae in giraffes elongated 29% faster than their lumbar and thoracic counterparts.

Vertical growth in giraffes is not solely dependant on neck elongation (Slijper, 1946; Mitchell & Skinner, 2003a), but also on elongation of the limbs and especially the metapodial bones more than the proximal limb bones (McMahon, 1975).

Growth curves of the shoulder height of giraffes (*Fig 1.1*) show clearly how fast these animals grow vertically (Hall-Martin, 1975) and also that much of this accelerated growth occurs within the first six to eight years of life.

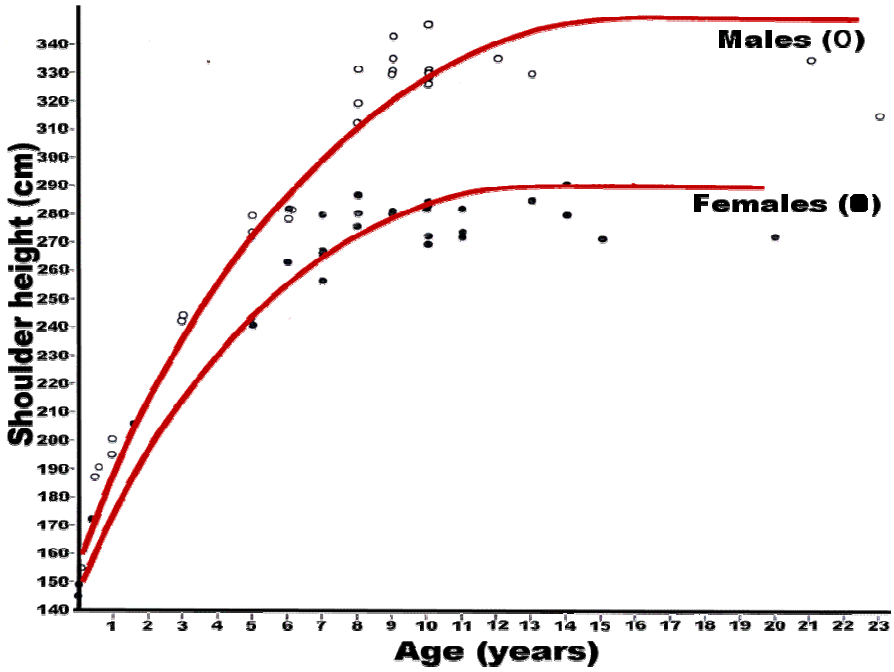


***Fig 1.1**: Shoulder height of giraffes (Hall-Martin, 1975)*

From this graph it is clear that, during the first six years of life, shoulder height in giraffe bulls increases by 120cm, whereas in the subsequent six year period the increase is only 60cm. Nevertheless, the giraffe growth curve follows the general form of all mammalian curves pertaining to mass, height, length and chest girth (Hall-Martin, 1975)

Evolutionary theories for the long neck and legs of the giraffe have been widely debated and vary from the long-familiar Darwinian argument of access to a non-competitive food resource to that of sexual selection (Simmons & Scheepers, 1996), thermoregulation (Brownlee, 1963) and even as a tool to avoid predation (Pincher, 1949; Brownlee, 1963). Whatever the function of the elongated legs and neck of the giraffe ultimately is, it has serious cardiovascular (e.g.

Warren, 1974; Badeer, 1988; Mitchell & Hattingh, 1993; Mitchell & Skinner, 1993; Pedley *et al*., 1996), respiratory (Mitchell & Skinner, 1993) and biomechanical (McMahon, 1975; Mitchell & Skinner, 2003a) consequences for the animal, to all of which it seems to have adapted.

Biomechanical considerations regarding the skeletal shape

The giraffe shape is unique among terrestrial mammals, with not even the okapi *Okapi johnstonii*, its closest relative, resembling it regarding height and slender appearance. Body mass, gravity and an elongated form will expose the giraffe skeleton, and especially the tubular limb bones, to greater loading forces.

Currey & Alexander (1985) proposed the following requirements for an optimal tubular bone:

- "That it be strong enough not to yield, under the greatest bending moments likely to act on it;
- that it be strong enough not to fail by fatigue, under the bending moments expected to act repeatedly on it ;
- that it be strong enough not to fracture, under the greatest bending moments likely to act on it;
- that it be stiff enough in bending; and
- that it be strong enough in bending under impact loading."

These qualities would be dependant on various factors, e.g.:

- composition of the bone matrix (Gaynor Evans, 1973; Pauwels, 1980)
- length of the bone (in relation to)
- diameter (outer and inner) and cross-sectional area of the bone (McMahon, 1975; Cloudsley-Thompson, 1976; Alexander, 1977 & 1982; Alexander *et al*., 1979; Currey & Alexander, 1985; Bertram & Biewener, 1988; Selker & Carter, 1989)
- angle of the longitudinal axis of the bone with the ground force (Biewener, 1983, 1989, 1990 & 1991)
- bone curvature (Bertram & Biewener, 1988 & 1992) and
- geometrical shape of the bone (Selker & Carter, 1989).

Almost all of the studies by the above mentioned authors propose mathematical predictions for these morphological characteristics, to only few of which the giraffe seems to conform (eg the reduced bone curvature of the giraffe femur). However, examining the survival and success of the species, it is apparent that the giraffe tubular bones must meet all the basic requirements set out by Currey & Alexander, notwithstanding their deviation from the general mathematical forecasting.

The cross-sectional area of the long bones of mammals is directly proportional to the square of their length (Cloudsley-Thompson, 1976). Consequently, slenderness in form is normally associated with a

decreased body mass (McMahon, 1973). Cross-sectional area normally increases as the mass of the animal increases, giving heavier animals, such as an elephant, a characteristic stocky appearance. However, in giraffes this seems not to be the case, since their metapodials, although having similar or even slightly greater cross-sectional areas than animals of similar mass, are much longer than predicted for their specific cross-sectional area (McMahon, 1975; Hamilton, 1978; Geraads, 1986).

Nevertheless, cross-sectional area is not the only mechanism for bone to adapt to higher loading forces due to elongation. Pauwels (1980) showed that in humans, bone would become denser at a point of increased stress, and density could therefore also act as a mechanism for increasing strength, as is beautifully explained in the wood and steel example of Gaynor Evans (1973), where he shows the difference in strength between similar volumes of these materials. In some preliminary measurements made by Mitchell & Skinner (2003a), they found the giraffe limb bones to be more dense than other parts of the skeleton, whilst the cervical vertebrae were found to be less dense than other vertebrae.

On the other hand, tubular bones could also increase their wall thickness as a means of withstanding higher loading forces (Currey & Alexander, 1985), albeit having a definite but small effect on total bone strength.

Disregarding direct adaptations of bones, there are also other mechanisms for reducing the total loading forces on bone, for example reducing the horizontal component of a loading force and therefore diverting most of the stress axially, directly through the bone matrix, by decreasing the angle of the bone with the vertical (Biewener 1983; 1989). Gaynor Evans (1973) showed that bones are much stronger in the axial than perpendicular plane, and therefore by making the axial component of the loading force greater and decreasing the perpendicular force, a bone would in fact become relatively stronger. Gambaryan (1974), although attributing it to facilitating access to high browse, stated that the giraffe's skeleton is characterized by straightening of most of its leg joints in the support phase. In a completely straight hind limb the sum of the knee and hock joint angles would be 360° - in the giraffe this is rarely less than 250°, while in other ungulates the norm in the support phase is usually less than 200°. This evidence supports the former statement regarding bone strength in the axial plane.

Apart from the total angle that the limb forms with the ground, a specific bone could also be curved to a certain extent. Although this bending has biomechanical function relating to the forces muscles exert on bones, it will ultimately weaken the bone along the line through its articulating surfaces (Biewener, 1983). With an increase in body mass of a species this bone curvature decreases allometrically. In giraffes,

however, this curvature (the deviation from the line connecting the centre of the bone's two articulating surfaces) seems to be especially small, with it being only 1.49mm in the femur compared to an average of 7.3±4.2mm in 10 other artiodactyls (Biewener, 1983). The giraffe's fairly straight bones can therefore also act as a mechanism to relatively strengthen its bones under increased vertical loading.

Furthermore, bone geometry, both in total and in cross section, also plays a role in the handling of high loading forces. An example of this is the longitudinal curvature of a long bone decreasing with an increase in mass (Bertram & Biewener, 1988) and the widening of epiphyses to distribute weight more evenly (Pauwels, 1980). Ultimately, as in all biological systems, bone strength is derived from a complex interplay of all the abovementioned factors, both intrinsic and extrinsic.

Just as the slenderness of the giraffe limbs poses a potential weak point in their skeletal integrity (according to mammalian norms), the elongated neck poses other biomechanical challenges. Hall-Martin *et al*. (1977) estimated the head and neck mass of mature bulls to be up to 250kg. This is close to one quarter of the total body mass and without some biomechanical adaptation, would seriously compromise manoeuvrability of the animal. But, although of great mass, the neck of the giraffe is relatively much lighter than expected when compared with other mammals. For example, the cervical component of the giraffe's *M. serratus ventralis* only constitutes 12% of the entire mass of the

muscle, while in other ungulates it comprises 35-45% of the total muscle body (Gambaryan, 1974).

In addition, Solounias (1999) showed an anatomical adaptation of the position of the neck in relation to the point of the shoulder (*Fig 1.2*). Although he ascribes this anomaly to a mechanism for allowing the giraffe to breathe whilst drinking, it could just as easily act as a biomechanical adjustment to ameliorate the carriage of a heavy neck.

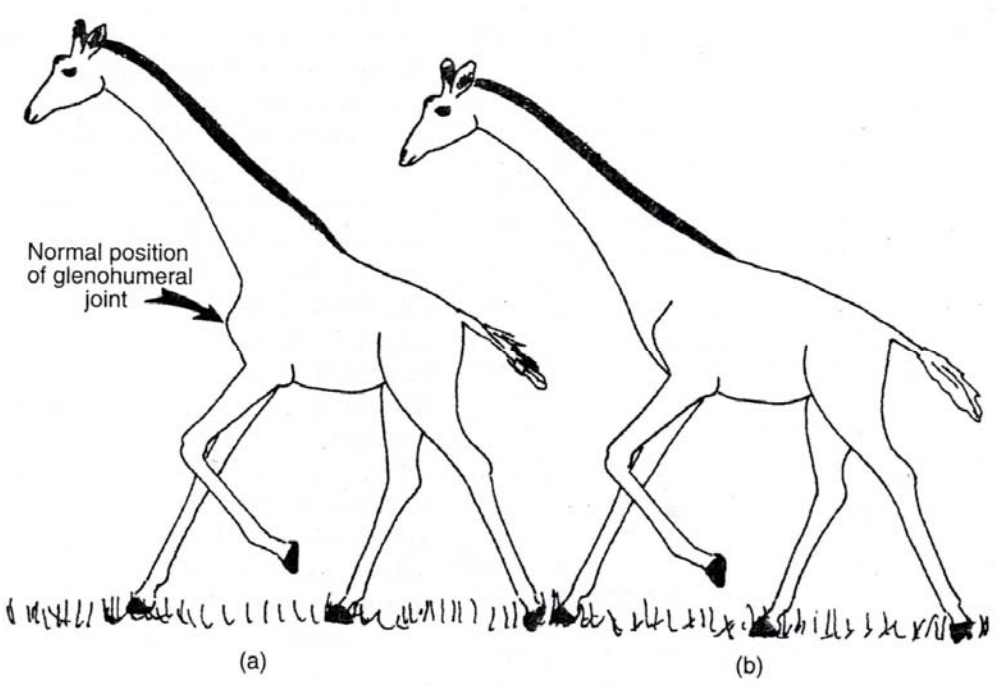


Fig 1.2: *(a) The actual giraffe conformation and (b) an imaginary giraffe with the neck in the typical ruminant position. (Solounias, 1999)*

Moreover, the head of the giraffe bears large sinuses and relatively small cranial appendages compared to prehistoric forms (Colbert, 1935; Mitchell & Skinner, 2003a) and other large artiodactyls like

buffaloes, both features of which result in a relatively less heavy head and thus improving biomechanical advantage for movement of the neck. Similarly, lighter (and therefore less dense) cervical vertebrae would also benefit manoeuvrability of the giraffe neck (Mitchell & Skinner, 2003a).

On the other hand, a relatively heavy neck could also be advantageous. For example, Dagg & Foster (1976) described how the neck is used as a counterbalance during galloping, while Coe (1967) did much work on the use of the neck as a weapon in the characteristic "necking" behaviour of giraffes. In both instances a reasonably heavy neck, and also relatively strong vertebrae, could ultimately favour the survival of the animal.

Furthermore, anatomically and mechanically, disregarding length, the neck of the giraffe has some unique features, like its lack of bony prominences for muscle insertion (Lankester, 1908; Gambaryan, 1974) which partly explains the ligamentum nuchae dominated control over lowering and lifting of the neck and head (Lankester, 1908; Mitchell & Skinner, 2003a).

Proportion of body mass that is skeleton

Skeletal mass is ultimately determined by the amount of minerals absorbed and accumulated during a lifetime. Of these skeletal minerals, Ca and P constitute the greatest proportion and could therefore become limiting factors in the growth and health of the skeleton and hence the success of the animal.

Mature bone is comprised of three main constituents: an organic base (38%), an inorganic fraction (or mineral component) (32%) and water (30%), with the mineral component consisting of mainly elementary hydroxyapetite crystals ($Ca_{10}(PO_4)_6(OH)_2$) (Georgievskii, 1982). Ca and P generally constitute 36% and 17% respectively of the mineral component (Underwood & Suttle, 1999), and therefore approximately 11.5% and 5.5% of the total bone mass, giving a Ca:P ratio of about 2.1:1.

Hall-Martin *et al*. (1977) estimated the skeletal component of the giraffe carcass to be 23.9% in mature bulls (equivalent to about 280kg), which is considerably higher than the 15-20% norm in cattle *Bos Taurus* (equivalent to about 150-200kg) (Simoes & Mira, 2002; Muldowney *et al*., 2001; Steen & Kirkpatrick, 1995). Accurate measurements of the total skeletal mass of giraffes have not yet been made, but Hall-Martin *et al.* (1977) estimated it to be 250kg. Mitchell & Skinner (pers comm) have weighed a dry skeleton of an immature, 3-year-old, female giraffe and found that it had a mass of 72.5kg, corresponding to a wet mass of

about 220-240kg. Moreover, if Ca content is approximately 20% of dry mass (Langman, 1978; this study) then this skeleton contained 4.5kg of Ca. If these estimates are accurate, the absolute amount of Ca (and P) required by giraffes will be proportionally higher than it is in cattle. Another calculation estimated that daily Ca absorption over a five-year growth period must average 20g in giraffes (Mitchell & Skinner, 2003b), to provide sufficient calcium.

That giraffes have difficulty in assimilating this amount of Ca can be inferred from an apparently highly variable serum concentration of Ca and P, which is summarized in the table (*Table 1.1*) below, with buffalo values shown for comparison. However, serum Ca and P levels are not good indicators of bone mineral status (Underwood & Suttle, 1999), but such values do give an indication of the labile nature of these minerals in the bloodstream. Both bone density and bone mineral content is commonly used as an indicator of both chronic and acute mineral status (Underwood & Suttle. 1999). Rib biopsies are the most common method used in live animals to determine their Ca and P status (Little, 1972; Little & Ratcliff, 1979, Read *et al*., 1986).

Table 1.1: *Plasma Ca and P concentrations (mmol/l) in the giraffes*

Reference	Giraffe (mmol/l)			Buffalo (mmol/l)		
	n	Ca	P	*n*	Ca	P
Sikes, 1969	4	3.5	1.3	8	2.3	1.5
Rossof, 1972	1	2.4	2.4	-	-	-
Drevemo *et al*. 1974	4	3.0±0.6	1.6±0.3	12	2.1±0.7	1.9±0.3
Rhodes, 1975	Not stated	2.1±0.2	2.0±0.3	Not stated	2.3±0.4	2.7±0.4
Bush *et al.*, 1980	14	2.6±0.4	3.2±0.9	-	-	-
	<1 month old *n* not stated	3.1±0.2	-	-	-	-
	1month-1 year *n* not stated	2.5±0.1	-	-	-	-
	>1 year old *n* not stated	2.4±0.4	-	-	-	-
Clauss *et al.*, 1999	4	0.65-2.2	-	-	-	-

Mineral balance of the giraffe diet

There is considerable evidence that the dietary intake and requirements of Ca and P in giraffes are finely balanced. Anecdotal evidence is frequent observations of osteophagia in giraffes (Nesbitt-Evans, 1970; Wyatt, 1971; Western, 1971; Hall-Martin, 1975; Hampton, 2002). Osteophagia is regarded as a sign of mineral deficiency, especially P (Theiler *et al.*, 1924), and this conclusion has been applied to giraffe (Langman, 1978).

Sikes (1969) also reported alkaline phosphatase activity (usually a marker of bone turnover rate) in giraffe serum 1.7 times higher than it was in buffaloes. Bush *et al.* (1980) found significantly higher alkaline phosphatase in neonatal giraffe compared to juveniles and adults and

concluded that their bone turnover rate was high. Rhodes (1975) also measured alkaline phosphatase activity but his values are not consistent with other values and may be erroneous. Serum Ca:P ratios calculated from the data shown in *Table 1.1* range from 0.8:1 to 2.7:1 in giraffes and from 1.1:1 to 1.5:1 in buffaloes (discounting Rhodes's data), which also suggests more labile regulation of blood Ca and P concentration in giraffes.

Giraffes are highly selective browsers (Pellew, 1984) with a digestive system morphologically ideal for this type of diet (Hoffman & Matern, 1988). Pellew (1984) was the only worker amidst a large group researchers working on giraffe feeding ecology to have investigated the underlying reason(s) for this selectivity (Leuthold & Leuthold, 1972; Oates, 1972; Hall-Martin, 1974 & 1975; Hall-Martin & Basson, 1975; Van Aarde & Skinner, 1975; Sauer *et al*., 1977; Kok & Opperman, 1980; Sauer *et al*., 1982; Pellew, 1983 to mention but a few). He found a statistically significant positive selection for P by females in the wet season and throughout the year by bulls. Furthermore, he describes the P levels in the diet to be marginal in absolute terms and possibly deficient taking the high Ca levels into consideration.

Historical evidence that supports the idea of a potential for Ca and P deficiency, is that the transformation of the biome from forest to grassland in Asia and China, eight to six million years ago, is associated with the extinction of the giraffe there (Mitchell & Skinner,

2003a). One possible cause for this extinction could be that the nutrient supply, and particularly Ca, was deficient: the Ca content of grass is approximately 0.42% (Dougall *et al*, 1964). Preferred diets of the extant African giraffe is leguminous browse (Pellew, 1983; 1984), the Ca content of which is 1.9% and which has a Ca:P ratio of 7.7:1 compared to 2:1 for grass (Pellew, 1984; Taylor, 1989; Mitchell & Skinner, 2003a). Thus, for an equivalent dry mass intake, giraffes can obtain four to five times more Ca from browse than from grass. A Ca:P ratio above 7:1 in domesticated ruminants would result in clinical signs of P deficiency (McDowell, 1992; Underwood & Suttle, 1999). This would include osteophagia, which is a known symptom of P deficiency (Theiler *et al*., 1924). Apart from osteophagia and occasional geophagia, no apparent clinical signs of P deficiency have been documented in free-ranging giraffes.

Seasonally there is also a high variability in the chemical composition, including the mineral content, of the plants constituting the giraffe's diet (Dougall *et al*., 1964; Groenewald *et al*., 1967; Bonsma, 1976; Pellew, 1984). Grant *et al.* (1995) showed through faecal P content that veld P was at its lowest during the period July to September, the same period osteophagia is most commonly observed in mostly cows in Kruger National Park, South Africa (Bengis, pers comm) and Klaserie, South Africa (Hall-Martin, 1975).

Consequently, with the giraffe having a gestation period of around 15 months (Dagg & Foster, 1976; Skinner & Smithers, 1990) and lactating for a similar period, cows would inevitably go through very challenging periods of mineral homeostasis during late pregnancy and lactation due to the high demands (especially for Ca) of these phases, and especially if these occur during April to October when browse availability is at its lowest.

Apart from the statistically proven selection for P (Pellew, 1984), another possible case of selection for P could be speculated upon from a study by Du Toit *et al.* (1990), where it was found that giraffes would selectively feed on a specific *Acacia nigriscens* tree, whilst ignoring nearby similar species. The result of this selective increased utilisation is amongst others, an increase in the P content of the plant material. This is mainly due to increased sprout production of which giraffes would utilise up to 85%, a figure so high that it could sometimes be to the detriment of the tree (Pellew, 1984b; Bond & Loffel, 2001). As a mechanism for increasing P intake, this is only speculative, as the study by Du Toit *et al*. was not based on giraffe feeding ecology, but rather on the ecology of *Acacias* in relation to herbivory.

The buffalo as the "conventional artiodactyl"

A full-grown African buffalo bull can weigh up to 800kg (Skinner & Smithers, 1990), which can be regarded as being of the same order as the 1174kg of its giraffe counterpart (Hall-Martin *et al*., 1977). Apart from their relation in mass and the fact that in both, body weight is concentrated over the forequarters, the anatomy of the giraffe and buffalo differ considerably. The giraffe bull stands two meters high at the shoulder compared to the 1.5m of the buffalo bull (Hall-Martin, 1975; Skinner & Smithers, 1990). The difference in the shape of their bodies is obvious, with the buffalo having the conventional bovid shape and the giraffe having a unique tall and slender build. The short stocky legs of the buffalo are in agreement with the principles of McMahon (1975) and Alexander (1977) on the relation of body mass to limb length and diameter.

Buffaloes have a digestive system extremely well adapted to the digestion of fibre (Hoffman, 1989), which makes them very efficient grazers (Sinclair, 1977; Taylor, 1989). Their grass diet contains a Ca:P of around 2:1 (Mitchell & Skinner, 2003a), which is in stark contrast with the browse utilised by giraffes (7.7:1 – Pellew, 1984; Mitchell & Skinner, 2003a).

Their similar mass, but more conventional vertical growth rate, shape and diet makes the African buffalo an ideal model for comparison to the

giraffe if differences with regards to skeletal morphology and physiology exist.

With this background in mind, this study was designed to establish if giraffes had any unique features regarding their bone density, skeletal calcium (Ca) and phosphorus (P) content and certain femoral and metacarpal morphological characteristics, compared to another herbivore, the African buffalo.

CHAPTER II

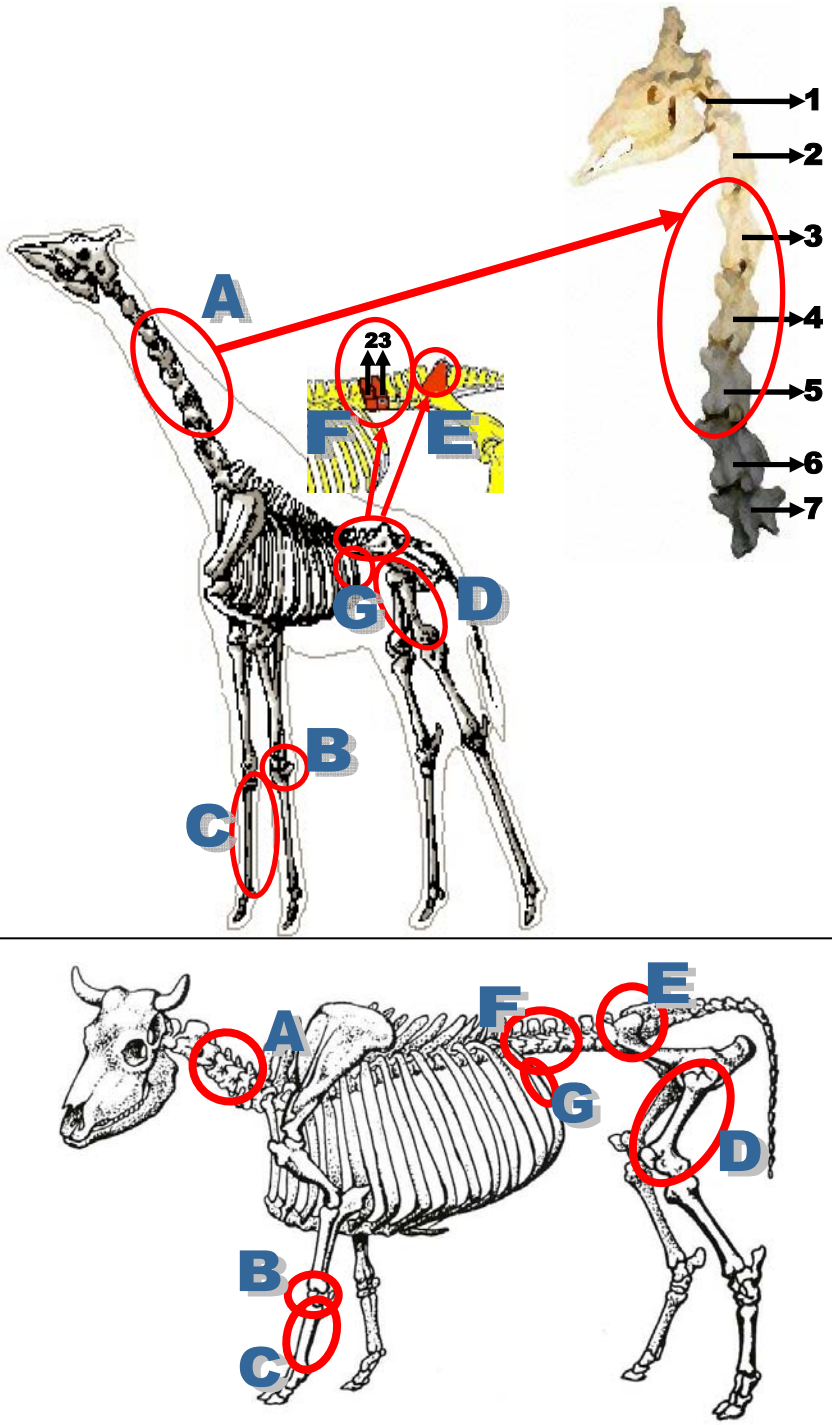
MATERIALS & METHODS

2.1 STUDY AREA & SAMPLE COLLECTION

Carcasses of mature giraffe and buffalo bulls were collected from predator kills and hunting/culling operations in and adjacent to Kruger National Park, South Africa. Only bulls older than six years were collected (i.e. all permanent incisors/canines were erupted (Hall-Martin, 1976, Taylor, 1988)). The specific age of each animal was unknown. Carcasses varied in age from 1-14 days depending on when deaths were reported and how accessible the carcass was. Field rangers, researchers, hunters and farmers in the area were notified of the project and asked to report finding any suitable carcasses. A diagram (*Fig 2.1*) was sent to these people to help them identify bones of carcasses (to determine suitability).

The following bones were collected from each carcass:

- third to fifth cervical vertebrae (*C3, C4, C5*),
- second and third lumbar vertebrae (*L2, L3*),
- Proximal arch of the 12th rib (*Rib*) (±10cm),
- Tuber coxa (*Tc*),
- Proximal (*Fp*) and distal (*Fd*) heads, and the mid-shaft (*Fs*) (±5-10cm) of the femur,
- Radial (*rC*), intermediate (*iC*), and ulnar (*uC*) carpals
- Mid-shaft of the metacarpus (*Mc*) (±5-10cm).



A - ***Cervical vertebrae***: **3rd, 4th, 5th neck vertebrae / 3e, 4e, 5e nekwerwels (NB 1st bone small / 1ste een klein!)**
B - ***Carpus***: **"Knee joint" of front leg (small bones) / "Knie gewrig" van voorbeen (klein beentjies)**
C - ***Metacarpus***: **Lower front leg (long bone) / Laer voorbeen (lang been)**
D - ***Femur***: **Upper back leg (large bone) / Agterste bobeen (groot been)**
E - ***Tuber coxa***: **Upper wing of hip bone / Boonste vlerk van heupbeen**
F - ***Lumbar vertebrae***: **2nd & 3rd vertebrae of back / 2e & 3e rugwerwels (lende)**
G - ***Rib***: **Piece of last rib next to vertebrae / Stuk van laaste rib langs rugwerwels**

Fig 2.1: *Diagrams used to help field rangers and other assistants identify bones (to establish whether carcass was suitable for collection)*

No distinction according to ecological/geological zones was made in the collection of samples. The quality of the veld also deteriorated considerably during the six month period of collection (April to September 2003). The effects (if any) of these variables on bone density, morphology, or mineral content could not be quantified.

2.2 DENSITY DETERMINATION

All soft tissue was removed from bones by dissection (*Fig 2.2*). Bones were not boiled because of the risk of Ca/P salt formation in water and thus alteration of their mineral content. Thereafter, bones were air-dried to constant mass, and weighed using a *Richter Scale KA-10* (Kubota Ltd, Pretoria, 1g accuracy).

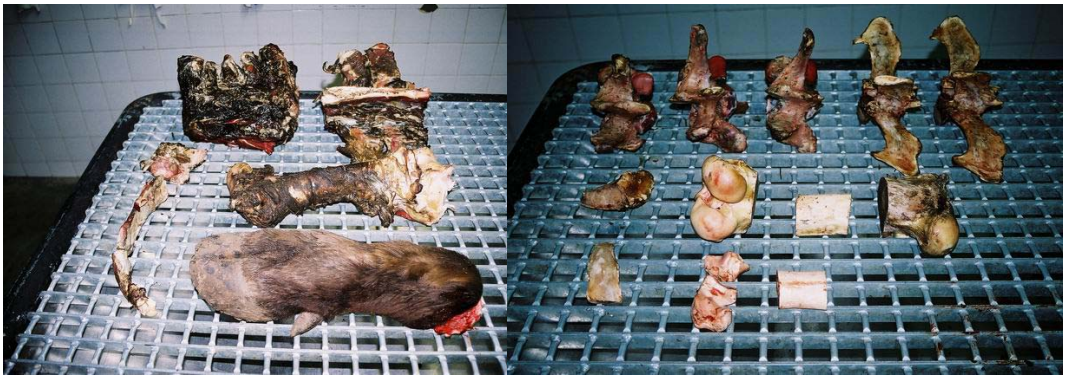


Fig 2.2: *Pictures of a set of buffalo bone samples before(left) and after (right) dissecting off all soft tissue*

According to studies done by Brain (1981, pers comm.) and Benzie *et al*. (1955; 1959), there was a significant difference in the density of different parts of long bones of sheep *Ovis aries* and indigenous goats

Capra hircus. It is for this reason that long bones in the study were divided into epiphyseal and diaphyseal parts to separate parts of dissimilar densities.

The volume of each sample was determined by the displacement of water using two custom-made containers (*Fig 2.3*) of different sizes. The one had an inside diameter of 10cm and a depth of 37cm (used for smaller bones) while the bigger one had an inside diameter of 25cm and a depth of 50cm.

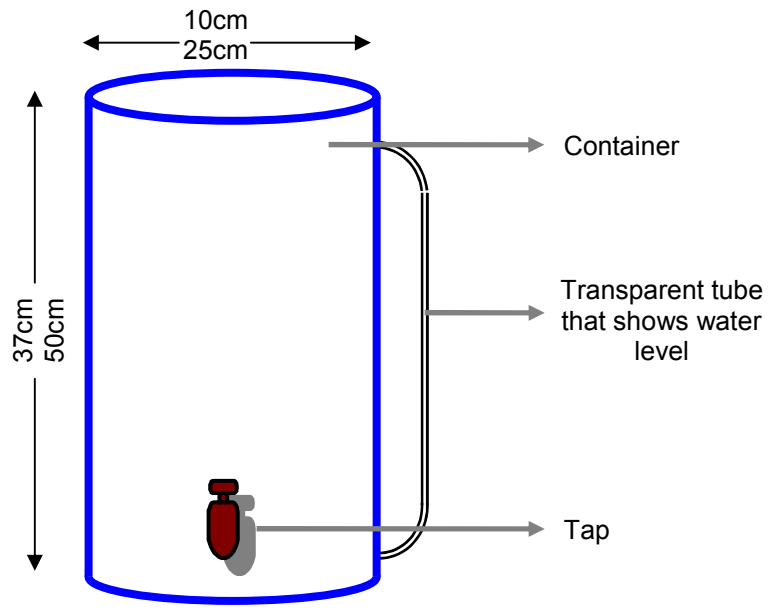


Fig 2.3: *Diagram of custom-made container used to determine volume of bones*

The container was filled with water at a temperature of about 22°C, to a set level. This level was marked on the transparent tube. The bone or bone fragment to be sampled was then placed in the container, and submerged. The volume of water displaced caused the water level in the transparent tube to rise. Water was let out of the container

immediately (i.e. bones were not allowed to soak) via a tap (see *Fig 2.3*) until the water level in the transparent tube returned to its original level. The amount of water drained was collected and weighed. The containers were calibrated using a wooden block of 1000cm^3 (10cm x 10cm x 10cm). The error was small enough to be ignored for the precision levels of the present study (0.05cm^3).

According to a study by Khan *et al.* (1997) the density of water at 0ºC is 0.9998395 g/ml, while at 20°C and 25°C it was 0.9982041 and 0.9970449 g/ml respectively. The temperature, at which the density of water is closest to 1 g/ml, is 4°C when it reaches 0.9999720 g/ml. In this study the density of water was rounded off to 1g/cm^3, thus the displaced mass of water equals the displaced volume. The volume determination of each bone was repeated 14 times to derive the mean volume. Mass density (Gaynor Evans, 1973) was calculated by dividing mass by volume and recorded as g/cm^3. Relative density (%) was determined by comparing the density of all bones to that of the mid shaft of the femur (taken to be 100%).

2.3 MINERAL ANALYSES

All samples were autoclaved at 250°F for 15 min at 15lb pressure in an *Almor P-09A* autoclave (Almor Ltd) at the State Veterinary office, Skukuza. This was done for disease control purposes, since the bones had to be moved from a Foot-and-Mouth disease (FMD) infected zone to a FMD free zone. Moreno & Forriol (2002) determined that autoclaving had no significant effect on the Ca and P composition of bones.

The following samples were used in mineral analyses: *C3, C4, C5, L3, Fp, Fs, rC, Mc, Tc* and *Rib*. Each bone was sawn into smaller pieces (±6-8cm^3) and defatted with stabilized trichlorethilene (TCE) for 48h in a *Degreasing Plant* (Proctor Industrial Cleaning Systems, Midrand) at the Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria. Thereafter these smaller pieces were ground to a coarse powder using a custom-made iron pestle and mortar. This coarse powder was then ground with a motor-driven mill (Mikro-Feinmühle-Culatti MFC, Janke IKA®-Labortechnik, 50/60Hz, 200W) to ±1mm^3. at Nutrilab, Department of Animal and Wildlife Sciences, Faculty of Natural and Agricultural Sciences, University of Pretoria.

0.5g (±0.005) of each sample was weighed in duplicate and wet ashed using Nitric and Perchloric acid at 230°C as described by Horwitz (2002). Thereafter each sample (in duplicate) was diluted 250 times

with distilled water and divided for Ca and P determination. P samples were diluted a further 10 times with distilled water before analyses and Ca samples 100 times with Lantanechloride ($LaCl_2$ 0.5%) to minimise interferences by P on the spectrophotometer.

Ca concentration was measured with an *Atomic Absorption Spectrophotometer 5100PC* (Perkin-Elmer) using the AOAC official method 935.13 (Horwitz, 2002). P was measured using an *Auto Analyser II* (Technicon^{TM}) according to the AOAC official method 965.17 (Horwitz, 2002).

The laboratory allowed an error of 10% between duplicates and for errors larger than this the analysis of that particular sample was repeated. The final result was obtained from the mean of the duplicates. Results for both minerals were generated as g/100g (%) and then converted to g/g, and also to g/cm^3 using the density determined earlier.

2.4 CROSS SECTIONAL AREA MEASUREMENTS

Femur and metacarpus shafts were transected perpendicular to the shaft at the mid-point of the middle third. The total cross-sectional (CSA) and marrow cross-sectional areas (MA) were estimated by drawing their outlines on graph paper and determining surface area (cm^3) by inspection. Bone area (BA, cm^3) at the mid-shaft was calculated by subtracting MA from CSA. Radius (cm) of the mid-shaft was calculated by assuming that the mid-shaft was cylindrical and using the equation $A = \pi r^2$. Similarly bone wall thickness (cm) was calculated by subtracting outer radius from inner (bone marrow) radius. Relationships between MA:CSA, and BA:CSA (%) were also determined.

2.5 DATA ANALYSES

An independent, two-tailed Student's t-test was used to determine significance of differences between results both inter- and intraspecifically.

Pearson's product moment correlation coefficient was used to determine correlations between different variables. For both types of statistical analysis P values of less than 0.05 were regarded as significant.

CHAPTER III

RESULTS

3.1 CARCASSES & BONES

Bones were collected from seven giraffe and nine buffalo carcasses. One of the giraffe carcasses had been frozen for two months prior to collection and this treatment decreased bone density by between 5 and 25% relative to the mean density of the rest of the giraffe bones. These bones (frozen) were discarded, as were some individual bones from other carcasses that were damaged by predators or bullets in such a way that their density could not be accurately determined. *Fig 3.1* depicts a typical collection site for giraffes. *Table 3.1* summarizes the relevant information pertaining to each carcass and *Fig 3.2* shows the location of each collection site.

Fig 3.1: *Typical giraffe collection site*

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Table 3.1: *Carcass collection information*

Carcass nr	Species	Locality	Collection date	Approximate date of death	Cause of death	Body conditon	Collector	Bones discarded
Gc01	Giraffe	23^{0}59'44"S 31^{0}48'03"E	05-05-2003	23-04-2003	Lions	Unknown	LvS	
Gc02	Giraffe	24^{0}32'31.8"S 31^{0}11'18.1"E	16-05-2003	16-03-2003	Shot (boma injury)	Unknown	LvS	All (frozen)
Gc03	Giraffe	24^{0}32'31.8"S 31^{0}11'18.1"E	27-05-2003	25-05-2003	Shot (cull)	Excellent	LvS	
Gc04	Giraffe	24^{0}13'55.8"S31^{0}38'03.0"E	31-05-2003	21-05-2003	Unknown	Unknown	LvS	*Fd*
Gc05	Giraffe	24^{0}32'31.8"S 31^{0}11'18.1"E	24-08-2003	24-08-2003	Shot (cull)	Excellent	LvS	
Gc06	Giraffe	24^{0}21'48.6"S 31^{0}55'01.7"E	01-09-2003	28-08-2003	Unknown (fell??)	Unknown	LvS	*Fd*
Gc07	Giraffe	24^{0}44'34.3"S 31^{0}44'04.5"E	01-09-2003	28-08-2003	Lions	Unknown	LvS	
Sc01	Buffalo	23^{0}33'21.1"S 31^{0}26'27.5"E	28-05-2003	27-05-2003	Shot (injured)	Excellent	LvS	*L2, L3, Fp, Fs, Fd, Tc*
Sc02	Buffalo	24^{0}54'04.7"S 31^{0}45'15.4"E	03-06-2003	29-05-2003	Lions	Unknown	LvS	
Sc03	Buffalo	24^{0}57'46.6"S 31^{0}26'53.7"E	18-06-2006	18-06-2003	Shot (disease control)	Excellent	SvD	*C3, C4, C5*
Sc04	Buffalo	25^{0}22'24"S 31^{0}58'51"E	27-06-2003	25-06-2003	Shot (cull)	Excellent	LvS	
Sc05	Buffalo	24^{0}23'46"S 31^{0}46'34"	13-07-2003	13-07-2003	Lions	Excellent	LvS	
Sc06	Buffalo	24^{0}59'26.7"S 31^{0}35'06.2"E	12-08-2003	12-08-2003	Boma cull (Brucella)	Excellent	AtD	
Sc07	Buffalo	24^{0}28'49.8"S 31^{0}38'47.4"E	13-08-2003	13-08-2003	Lions	Excellent	LvS	
Sc08	Buffalo	24^{0}32'31.8"S 31^{0}11'18.1"E	25-08-2003	16-08-2003	Shot (hunt)	Good	LvS	
Sc09	Buffalo	24^{0}32'00"S 31^{0}45'36"E	18-08-2003	19-08-2003	Lions	Unknown	JW	

LvS: Louis van Schalkwyk

SvD: Schalk van Dyk

AtD: At Dekker

JW: Julie Wolhuter

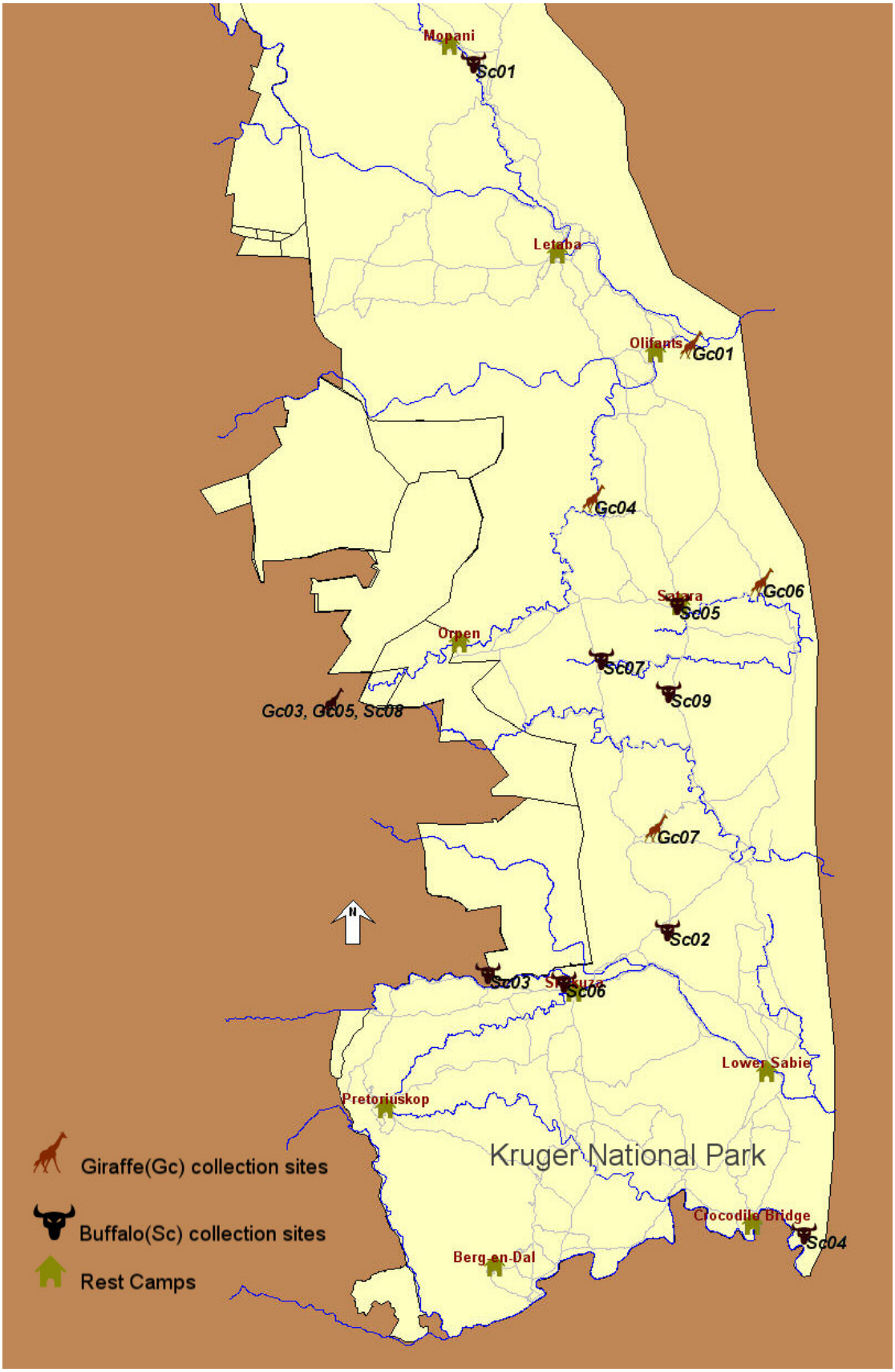

Fig 3.2: *Map of Kruger National Park and adjacent reserves showing locations of collection sites*

3.2 BONE DENSITY

The average (±SD) mass, volume and density of each sample are listed in *Table 3.2 & 3.3* for giraffes and buffaloes respectively. In both species the *Fs* and *Mc* were the densest bones measured, with the buffalo *Fs* being the densest of all the bones at 2.0±0.1g/cm^3 and the giraffe *Mc* being the densest of the giraffe bones at 1.9±0.1 g/cm^3. The *Fs* and *Mc* of both species were also significantly (P<0.01) denser than any of the other bones measured. The order of descending density in giraffes is:

*Mc>Fs>>**uC>iC>rC>Rib>C3>L3>C4>L2>Fp>C5>Fd>Tc*

and buffaloes:

*Fs>Mc>>**uC>Rib>rC>iC>L3>C4>C3>L2>C5>Fd>Fp>>*Tc*

(with >> referring to a significant (* P<0.05 and ** P<0.01) change in density).

The general order of grouping according to density was the same for both species: long bone shafts>>carpal bones>>rib>>vertebrae & femur heads>>tuber coxae.

Fig 3.3 depicts the relative bone density (relative to *Fs* as 100%) of the collected bones of both species. This graph is very similar to the graph showing absolute densities (*Fig 3.4*). The figures within each bar show the number of bones (*n*) used to determine each value.

Table 3.2: *Mean (±SD) mass, volume and density of giraffe samples*

Bone Sample	*n*	**Mass** (g)	**Volume** (cm^3)	**Density** (g/cm^3)
C3	6	1795.7 ±271.0	1308.0 ±24.3	1.4 ±0.1
C4	6	2215.0 ±307.8	1686.9 ±24.7	1.3 ±0.1
C5	6	2584.3 ±401.1	2011.0 ±20.5	1.3 ±0.1
L2	6	503.5 ±38.0	383.9 ±7.1	1.3 ±0.0
L3	6	521.2 ±52.2	390.5 ±9.0	1.3 ±0.1
Fp	6	1259.7 ±158.2	973.7 ±7.7	1.3 ±0.1
Fs	6	489.0 ±57.9	256.6 ±4.0	1.9 ±0.1
Fd	4	2559.5 ±661.8	2073.8 ±27.6	1.2 ±0.1
rC	6	171.2 ±25.4	116.0 ±2.7	1.5 ±0.1
iC	6	154.5 ±23.0	101.0 ±2.8	1.5 ±0.1
uC	6	149.7 ±25.2	92.2 ±2.1	1.6 ±0.0
Mc	6	568.8 ±118.4	297.6 ±3.7	1.9 ±0.1
Rib	6	116.8 ±39.1	80.6 ±3.4	1.5 ±0.1
Tc	6	218.2 ±86.4	182.8 ±2.9	1.2 ±0.1

Table 3.3: *Mean (±SD) mass, volume and density of buffalo samples*

Bone Sample	*n*	**Mass** (g)	**Volume** (cm^3)	**Density** (g/cm^3)
C3	8	522.4 ±108.7	378.3 ±4.2	1.4 ±0.1
C4	8	560.6 ±116.7	402.6 ±4.8	1.4 ±0.1
C5	8	567.3 ±118.9	415.9 ±3.9	1.4 ±0.1
L2	8	344.8 ±47.0	251.1 ±3.6	1.4 ±0.1
L3	8	374.3 ±61.1	268.3 ±3.8	1.4 ±0.1
Fp	8	691.4 ±111.3	555.6 ±4.8	1.3 ±0.1
Fs	8	199.3 ±31.8	102.1 ±1.2	2.0 ±0.1
Fd	8	811.6 ±116.7	640.4 ±3.8	1.3 ±0.1
rC	9	52.0 ±7.5	33.9 ±1.0	1.5 ±0.1
iC	9	36.8 ±3.2	24.6 ±1.0	1.5 ±0.2
uC	9	42.0 ±4.7	26.7 ±0.9	1.6 ±0.2
Mc	9	104.8 ±19.5	56.6 ±1.2	1.9 ±0.1
Rib	9	52.6 ±7.8	34.3 ±0.9	1.6 ±0.1
Tc	8	143.5 ±52.1	133.8 ±1.7	1.1 ±0.1

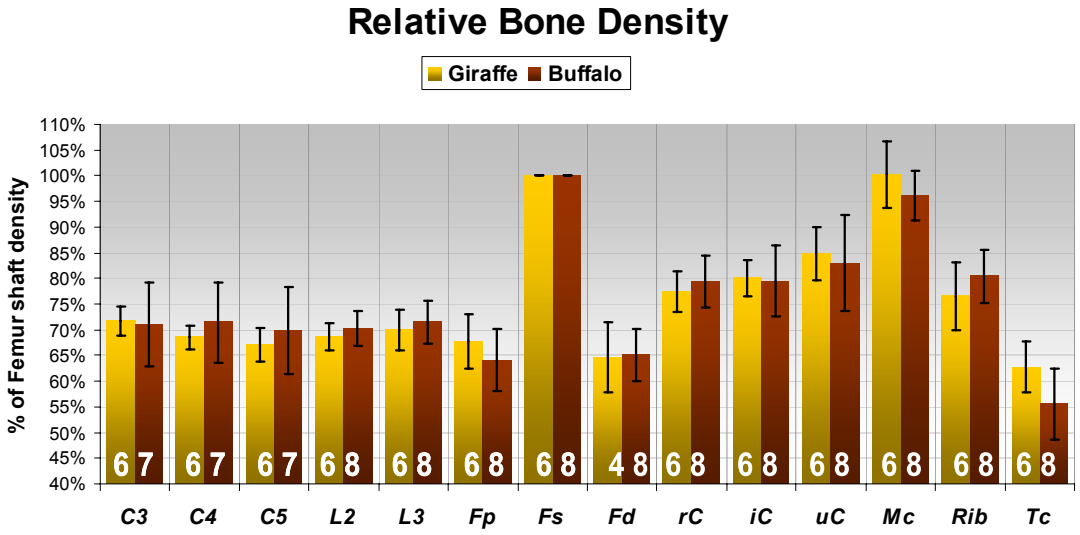

Fig 3.3: *Bone density relative to density of Fs*

What the relative density graph does show, is that the grouped density trend is similar amongst the bones of both species. *Mc* were as dense as *Fs*, while carpals and *Rib* were 80% as dense as *Fs*. Vertebrae and femur heads were 62-74% as dense as *Fs*. *Tc* in giraffes were almost as dense as vertebrae, while in buffaloes they were about 15% less dense than vertebrae.

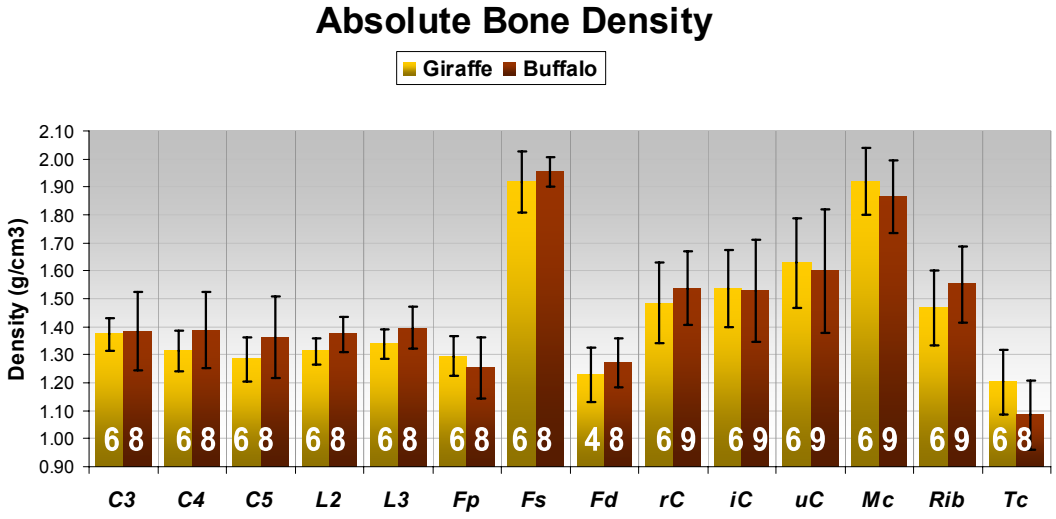

Fig 3.4: *Absolute bone density*

No significant differences (P<0.05) in bone density between specific bones of the two species could be found. *Table 3.4* shows the P-values for the significance of interspecific differences in density of each bone. The largest differences were between the second lumbar vertebrae and tuber coxae (P=0.070 and P=0.093 respectively), although still not significant at the P<0.05 level.

Table 3.4: *Interspecific significance (P) of difference in density*

Bone Sample	Giraffe	Buffalo	*P*
	N	*n*	
C3	6	8	*0.863*
C4	6	8	*0.249*
C5	6	8	*0.259*
L2	6	8	*0.070*
L3	6	8	*0.137*
Fp	6	8	*0.424*
Fs	6	8	*0.406*
Fd	4	8	*0.461*
rC	6	9	*0.485*
iC	6	9	*0.932*
uC	6	9	*0.801*
Mc	6	9	*0.435*
Rib	6	9	*0.251*
Tc	6	8	*0.093*

Table 3.5 & 3.6 illustrate intraspecific differences in bone densities. These differences varied from highly significant (P<0.001) between the long bone shafts (*Fs, Mc*) and vertebrae to nonsignificant between

vertebrae and femur heads. The most obvious differences between the two tables could be seen in the *Tc* and carpal bones.

Table 3.5: *Giraffe: Intraspecific bone density differences*

C4	***C5***	***L2***	***L3***	***Fp***	***Fs***	***Fd***	***rC***	***iC***	***uC***	***Mc***	***Rib***	***Tc***	
ns	*ns*	*ns*	*ns*	*ns*	***	*	*ns*	*	**	***	*ns*	**	***C3***
	ns	*ns*	*ns*	*ns*	***	*ns*	*	**	**	***	*	*ns*	***C4***
		ns	*ns*	*ns*	***	*ns*	*	**	***	***	*	*ns*	***C5***
			ns	*ns*	***	*ns*	*	**	***	***	*	*ns*	***L2***
				ns	***	*ns*	*	**	**	***	*ns*	*	***L3***
					***	*ns*	*	**	***	***	*	*ns*	***Fp***
						***	***	***	**	*ns*	***	***	***Fs***
							*	**	**	***	*	*ns*	***Fd***
								ns	*ns*	***	*ns*	**	***rC***
									ns	***	*ns*	**	***iC***
										**	*ns*	***	***uC***
											***	***	***Mc***
												**	***Rib***

ns Not significant * P<0.05 ** P<0.01 *** P<0.001

Table 3.6: *Buffalo: Intraspecific bone density differences*

C4	***C5***	***L2***	***L3***	***Fp***	***Fs***	***Fd***	***rC***	***iC***	***uC***	***Mc***	***Rib***	***Tc***	
ns	*ns*	*ns*	*ns*	*ns*	***	*ns*	*	*ns*	*	***	*	***	***C3***
	ns	*ns*	*ns*	*	***	*ns*	*	*ns*	*	***	*	***	***C4***
		ns	*ns*	*ns*	***	*ns*	*	*ns*	*	***	*	**	***C5***
			ns	*	***	*	**	*	*	***	**	***	***L2***
				**	***	**	*	*ns*	*	***	*	***	***L3***
					***	*ns*	***	**	**	***	***	*	***Fp***
						***	***	***	***	*ns*	***	***	***Fs***
							***	**	**	***	***	**	***Fd***
								ns	*ns*	***	*ns*	***	***rC***
									ns	***	*ns*	***	***iC***
										**	*ns*	***	***uC***
											***	***	***Mc***
												***	***Rib***

ns Not significant * P<0.05 ** P<0.01 *** P<0.001

When cervical vertebrae, lumbar vertebrae and carpals were grouped together, a significant interspecific difference (P=0.017) in the density of the lumbar vertebrae was found (*Table 3.7*).

Table 3.7: *Grouped vertebrae and carpal bone density comparison*

Bone sample	Giraffe	Buffalo	*P*
	Density ±SD	Density ±SD	
Cervical Vertebrae	1.3 ±0.1	1.4 ±0.1	*0.132*
Lumbar Vertebrae	1.3 ±0.0	1.4 ±0.1	*0.017*
Carpals	1.6 ±0.2	1.6 ±0.2	*0.913*

What is apparent from this table is the very similar densities of cervical and lumbar vertebrae within a species.

Cervical vertebrae, although of the same density, differed greatly in mass and volume between the two species (*Fig 3.5*). When giraffe cervical vertebrae were compared with each other it was found that they showed a decrease in mass and volume in a caudal direction. In volume they compared as follows:

$$C3 << ** C4 << * C5 >> *** L2 \approx L3$$

(With << referring to a significant change and ≈ referring to no significant difference) while in mass it was slightly different:

$$C3 << ** C4 \approx C5 >> *** L2 \approx L3.$$

For buffaloes the same comparison gave a totally different result for both mass and volume comparisons:

$$C3 \approx C4 \approx C5 >> *** L2 \approx L3.$$

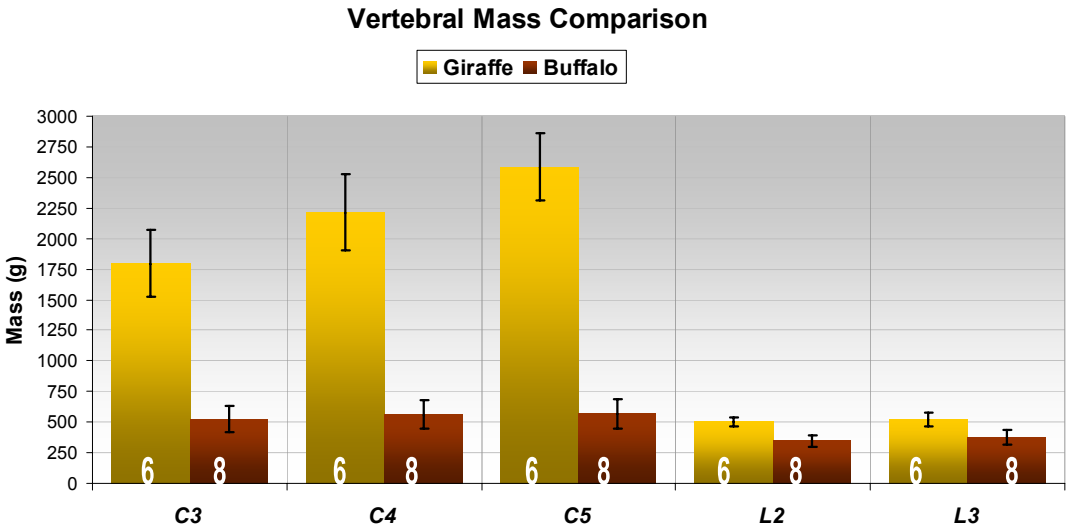

Fig 3.5: *Vertebral mass comparison*

Giraffe cervical vertebrae were on average 4-5 times heavier than their lumbar counterparts, while in buffaloes the cervical vertebrae were only 1.5 times heavier than the lumbar vertebrae. Volume measurements compared likewise in both species.

3.3 MINERAL CONTENT

Tables 3.8(a) and *3.8(b)* show the Ca and P concentration (g Ca or P/g bone) and the ratio of Ca:P in selected bones of 6 individual giraffes and 9 individual buffaloes. These tables also show Pearson's product-moment correlation coefficients between bone density and bone Ca, P, and Ca+P concentration.

Table 3.8(a): *Ca, P, Ca+P, Ca:P, and Pearson's correlation coefficients (r) between density and mineral content for all bones analysed in each of 6 giraffes*

Giraffe number	*n* bones	Ca g/g	P g/g	Ca+P g/g	Ca:P	Density	r Ca:D	r P:D	r Ca+P:D
1	9	0.203 ±0.02	0.097 ±0.01	0.300 ±0.03	2.09 ±0.15	1.43 ±0.25	0.772	0.880	0.811
3	10	0.185 ±0.02	0.094 ±0.01	0.279 ±0.03	1.97 ±0.10	1.35 ±0.23	0.761	0.934	0.836
4	10	0.194 ±0.01	0.094 ±0.01	0.288 ±0.01	2.07 ±0.10	1.47 ±0.27	0.860	0.861	0.891
5	10	0.193 ±0.02	0.092 ±0.01	0.285 ±0.02	2.10 ±0.06	1.51 ±0.23	0.743	0.677	0.831
6	9	0.195 ±0.02	0.095 ±0.01	0.290 ±0.02	2.05 ±0.12	1.52 ±0.32	0.142	0.442	0.249
7	10	0.204 ±0.02	0.096 ±0.01	0.300 ±0.02	2.13 ±0.09	1.50 ±0.28	0.504	0.762	0.602
ALL	58	0.196 ±0.01	0.095 ±0.02	0.290 ±0.01	2.07 ±0.06	1.46 ±0.06	0.686	0.763	0.809

The average Ca content of giraffe bones is 19.6 ± 1.0%, P is 9.5 ± 2.0% and Ca+P is 29.0 ± 1.0%. In buffaloes (*Table 3.8(b)*) equivalent values are 20.2 ± 0.6%, 9.5 ± 0.2%, and 29.7 ± 0.7%. Differences are not significant. However the Ca:P ratio of the 58 giraffe bones

analysed of 2.07 ± 0.06 is significantly lower (P<0.05, t=2.00) than it is in the 79 buffalo bones analysed (2.12 ± 0.07).

Table 3.8(b): *Ca, P, Ca+P, Ca:P, and Pearson's correlation (r) coefficients between density and mineral content for all bones analysed in each of 9 buffaloes.*

Buffalo number	N bones	Ca g/g	P g/g	Ca+P g/g	Ca:P	Density	r Ca:D	r P:D	r Ca+P:D
1	4	0.202 ±0.02	0.095 ±0.01	0.297 ±0.02	2.13 ±0.07	1.43 ±0.19	0.927	0.957	0.948
2	10	0.204 ±0.03	0.092 ±0.01	0.296 ±0.02	2.22 ±0.15	1.44 ±0.21	0.837	0.889	0.858
3	6	0.196 ±0.02	0.093 ±0.01	0.289 ±0.02	2.11 ±0.05	1.47 ±0.21	0.933	0.886	0.882
4	10	0.203 ±0.02	0.095 ±0.01	0.298 ±0.02	2.14 ±0.16	1.52 ±0.30	0.716	0.740	0.783
5	9	0.200 ±0.01	0.099 ±0.01	0.299 ±0.01	2.02 ±0.09	1.58 ±0.33	0.587	0.493	0.575
6	10	0.195 ±0.02	0.097 ±0.01	0.292 ±0.02	2.01 ±0.18	1.48 ±0.23	0.680	0.898	0.815
7	10	0.200 ±0.02	0.095 ±0.01	0.295 ±0.02	2.11 ±0.26	1.60 ±0.26	0.476	0.469	0.515
8	10	0.200 ±0.02	0.094 ±0.01	0.294 ±0.02	2.13 ±0.09	1.39 ±0.34	0.714	0.765	0.765
9	10	0.215 ±0.02	0.098 ±0.01	0.313 ±0.02	2.19 ±0.07	1.41 ±0.36	0.760	0.735	0.766
ALL	79	0.202 ±0.006	0.095 ±0.002	0.297 ±0.007	2.12 ±0.07	1.48 ±0.07	0.574	0.622	0.837

As the average P content of the bones of both species is identical (9.5%), the difference in ratios must result from variation in Ca content. In the giraffe bones studied, Ca content varied from 0.185 ± 0.02 g/g to 0.204 ± 0.02 g/g, with the lower value significantly different to the higher one (P<0.05, t=2.21). In buffaloes Ca content ranges from a low

of 0.195 ± 0.02 g/g to a high of 0.215 ± 0.02 g/g, and the differences in these values are also statistically significant ($P<0.05$, $t=2.3$). The variations of Ca and P in both species suggest that the Ca content of bones is more labile than their P content.

In the previous section it was shown that, between species, the average density of bones is not significantly different, although within a species density differs. Pearson's correlation coefficients show that changes in density are linked significantly to changes in Ca and P concentration (*Tables 3.8(a)* and *3.8(b)*, $P<0.05$). Not surprisingly the combination of Ca and P is a better predictor of density than either Ca or P alone. Ca and P together account for 65% of variation in density in giraffe bones and 70% in buffalo bones.

Within a species (*Tables 3.9(a), 3.9(b), 3.9(c), 3.9(d)*) the Ca and P content follow bone density findings closely. Bones supporting body mass (e.g. *Fs*, *Fp* and *Mc*) have high density and contain significantly more Ca and P than other bones, especially vertebrae. These tables also show interspecific differences between the bones. In two bones (*C5, L3*) the Ca concentration in giraffes is significantly lower than it is in buffaloes and in *Fp* it is higher. With respect to P content only *C5* and *Mc* appear to differ significantly between species (*Table 3.9(b)*).

Table 3.9(a): *Calcium concentration (g/g) in all bones analysed*

	C3	C4	C5	L3	Fp	Fs	rC	Mc	Rib	Tc	ALL
Giraffe	0.182	0.186	0.181	0.185	0.208	0.218	0.190	0.217	0.200	0.188	0.196
(N=6)	±0.02	±0.01	±0.02	±0.01	±0.01	±0.02	±0.01	±0.01	±0.01	±0.01	±0.01
Buffalo	0.195	0.195	0.197	0.204	0.195	0.233	0.198	0.220	0.206	0.176	0.202
(N=9)	±0.02	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.02	±0.006
t value	1.08	1.91	3.40*	3.22*	2.28*	1.56	1.51	0.526	0.759	1.20	0.857

*P<0.05

Table 3.9(b): *Phosphate concentration (g/g) in all bones analysed*

	C3	C4	C5	L3	Fp	Fs	rC	Mc	Rib	Tc	ALL
Giraffe	0.087	0.090	0.088	0.091	0.098	0.105	0.093	0.108	0.096	0.088	0.095
(N=6)	±0.007	±0.003	±0.005	±0.002	±0.004	±0.009	±0.006	±0.003	±0.002	±0.004	±0.020
Buffalo	0.090	0.090	0.094	0.094	0.097	0.110	0.094	0.104	0.098	0.084	0.095
(N=9)	±0.009	±0.008	±0.004	±0.004	±0.005	±0.002	±0.004	±0.004	±0.004	±0.007	±0.002
t value	0.19	-	2.0*	1.67	0.40	1.22	0.36	2.10*	1.18	1.29	0.667

*P<0.05

Table 3.9(c): *Ca+P concentration (g/g) in all bones analysed*

	C3	C4	C5	L3	Fp	Fs	rC	Mc	Rib	Tc	All
Giraffe	0.270	0.276	0.269	0.276	0.306	0.323	0.281	0.325	0.296	0.276	0.290
	±0.02	±0.01	±0.03	±0.01	±0.01	±0.03	±0.01	±0.02	±0.01	±0.02	±0.02
Buffalo	0.287	0.288	0.291	0.298	0.292	0.343	0.292	0.324	0.304	0.260	0.298
	±0.02	±0.02	±0.02	±0.02	±0.02	±0.01	±0.01	±0.01	±0.02	±0.02	±0.02
t value	1.00	0.10	1.40	2.63*	1.75	1.54	1.83	1.10	0.17	1.78	1.00

*P<0.05

Table 3.9(d): *Ca:P ratios for all bones analysed*

	C3	C4	C5	L3	Fp	Fs	rC	Mc	Rib	Tc	ALL
Giraffe	2.09	2.07	2.06	2.05	2.13	2.07	2.09	2.00	2.09	2.15	2.07
(N=6)	±0.13	±0.08	±0.12	±0.11	±0.84	±0.07	±0.09	±0.09	±0.14	±0.06	±0.06
Buffalo	2.19	2.18	2.10	2.18	2.03	2.12	2.10	2.11	2.11	2.10	2.12
(N=9)	±0.22	±0.21	±0.13	±0.12	±0.12	±0.12	±0.16	±0.18	±0.12	±0.14	±0.07
t value	1.67	2.75*	0.80	2.60*	0.27	0.96	0.20	1.47	0.29	0.89	2.00*

*P<0.05

Of the 14 bones analysed for density, eight were complete (*C3, C4, C5, L2, L3, rC, iC and uC*) and of these eight, Ca and P content was measured in five (*C3, C4, C5, L3, rC*) to establish if the mineral content of neck vertebrae of giraffe were unique (compared to a lumbar vertebra) and to establish if the mineral content of weight bearing bones was different. These five bones also allow a comparison and estimation of skeletal mass in the two species. The mass of *L3* and its Ca and P content is similar in both species and thus it serves as a reference for the other bones.

Analyses of some characteristics of the five bones is shown in *Tables 3.10(a), 3.10(b), 3.10(c), 3.10(d)* together with their total Ca and P content calculated from their mass and Ca and P concentration (g/g). Ca concentration (g/g) of neck vertebrae is not different to that of lumbar vertebra, despite their morphological differences described in the previous section. On the other hand, the Ca and P concentration of limb bones is significantly greater than it is in vertebrae. These tables also suggest that, although the mineral concentration (g/g) of bones in the two skeletons is similar, the skeletal mass of giraffes is probably at least three-fold greater than it is in buffaloes mainly because of the greater mass of the cervical and limb skeleton. Consequently total Ca and P content is approximately three-fold greater in giraffes. Body mass of mature bulls in the two species differs by about a factor of two (buffalo 603 ± 62kg; giraffe 1184 ± 70kg, Skinner & Smithers, 1990).

Thus giraffe have to accumulate significantly more Ca and P during growth.

Table 3.10(a): *Ca content of marker bones (g/g)*

	units	C3	C4	C5	L3	rC	ALL
Giraffe (N=6)	g/g	0.182 ±0.02	0.186 ±0.01	0.181 ±0.02	0.185 ±0.01	0.190 ±0.01	0.185 ±0.01
	Total mass (g)	1795.6 ±271.0	2215.0 ±307.8	2584.3 ±401.1	521.2 ±52.2	171.2 ±25.4	7287.3 ±1018.5
	Mass Ca (g)	327.3 ±57.9	412.5 ±63.5	472.3 ±117.6	96.4 ±10.4	32.1 ±4.6	1335.3 ±226.0
	Ca:P	2.09 ±0.13	2.07 ±0.08	2.06 ±0.12	2.05 ±0.11	2.09 ±0.09	2.07 ±0.10
Buffalo[#] (N=8)	g/g	0.195 ±0.02	0.195 ±0.01	0.197 ±0.01	0.204 ±0.01	0.198 ±0.01	0.198 ±0.01
	Total mass (g)	554.1 ±66.1	597.3 ±57.9	604.9 ±57.3	389.1 ±47.8	53.4 ±6.7	2189.2 ±208.8
	Mass Ca (g)	108.2 ±22.7	115.6 ±14.5	123.8 ±12.3	79.7 ±12.0	10.7 ±1.7	450.8 ±56.1
t value g/g		1.08	1.91	3.40*	3.22*	1.51	5.2*

*P<0.05
[#] excluding B#8

Table 3.10(b): *P content of marker bones (g/g)*

	Units	C3	C4	C5	L3	rC	ALL
Giraffe (N=6)	g/g	0.087 ±0.007	0.090 ±0.003	0.088 ±0.005	0.091 ±0.002	0.093 ±0.006	0.090 ±0.005
	Total Mass (g)	1795.6 ±271.0	2215.0 ±307.8	2584.3 ±401.1	521.2 ±52.2	171.2 ±25.4	7287.3 ±1018.5
	Mass P (g)	157.0 ±27.0	199.0 ±26.4	228.6 ±45.6	47.1 ±4.3	15.8 ±2.6	647.5 ±100.8
Buffalo[#] (N=8)	g/g	0.090 ±0.009	0.090 ±0.008	0.094 ±0.004	0.094 ±0.004	0.094 ±0.003	0.093 ±0.006
	Total Mass (g)	554.1 ±66.1	597.3 ±57.9	604.9 ±57.3	389.1 ±47.8	53.4 ±6.7	2189.2 ±208.8
	Mass (P) (g)	46.9 ±11.8	53.4 ±4.9	56.7 ±6.1	39.2 ±9.7	5.1 ±0.7	199.3 ±23.7
T value g/g		0.19	-	2.0*	1.67	0.36	1.0

*P<0.05
[#]excluding B#8

Table 3.10(c): *Ca+P content of marker bones*

	Units	C3	C4	C5	L3	rC	All
Giraffe	g/g	0.270 ±0.02	0.276 ±0.01	0.269 ±0.03	0.276 ±0.01	0.281 ±0.01	0.274 ±0.01
Buffalo	g/g	0.287 ±0.02	0.288 ±0.02	0.291 ±0.02	0.298 ±0.02	0.292 ±0.01	0.290 ±0.01
t value		1.00	0.100	1.40	2.63*	1.83	2.67

*P<0.05

Table 3.10(d): *Ca:P ratio in marker bones*

	C3	C4	C5	L3	rC	ALL
Giraffe (N=6)	2.09 ±0.13	2.07 ±0.08	2.06 ±0.12	2.05 ±0.11	2.09 ±0.09	2.07 ±0.10
Buffalo[#] (N=8)	2.21 ±0.24	2.17 ±0.23	2.11 ±0.14	2.20 ±0.10	2.11 ±0.17	2.16 ±0.17
t value	1.12	1.03	0.71	2.50*	0.25	2.57*

*P<0.05
[#] = excluding B#8

One of the buffalo (Sc08), although meeting our criterion of being more than six years old, had a skeleton that differed significantly from the others. It seemed to have approximately half the body and skeletal mass of the others. Analysis of this buffalo's skeletal characteristics (*Table 3.11*) shows no significant differences in Ca and P concentration (g/g) compared to the other buffaloes (*Tables 3.10(a), 3.10(b), 3.10(c), 3.10(d)*), but obviously the bones contain far less absolute amounts of Ca and P. These differences are most marked in cervical vertebrae. These findings suggest that bones supporting body mass accumulate Ca and P more quickly than do others, and that cervical vertebrae Ca and P content in buffaloes increases as the mass of the head, neck, and horns increases.

Table 3.11: *Skeletal characteristics of buffalo #8*

Mineral	Units	C3	C4	C5	L3	rC	ALL
	Total bone mass (g)	300	304	304	270	41	1219
Ca	Ca (g/g)	0.183	0.209	0.186	0.200	0.190	0.194 ±0.010
	Mass Ca (g)	54.9	63.5	56.5	53.9	7.8	236.5
P	g/g	0.088	0.094	0.091	0.099	0.093	0.093 ±0.004
	Mass P (g)	26.3	28.7	27.5	26.8	3.8	113.1
Ca:P		2.08	2.22	2.04	2.02	2.04	2.08 ±0.08

3.4 CROSS-SECTIONAL AREA AND BONE MORPHOLOGY

Table 3.12 shows the results of the total (CSA) and marrow cross-sectional areas (MA) of mid-shaft femurs (*Fs*) and metacarpi (*Mc*) measured, and compares these on an interspecific level.

Table 3.12: Comparison of interspecific differences in limb bone morphology in giraffes and buffaloes

		Giraffe	Buffalo	
	Measurement	**mean ± SD**	**mean ± SD**	**Significance**
Fs	CSA (cm^2)	33.9 ± 1.6	19.0 ± 1.8	***
	MA (cm^3)	9.3 ± 1.1	6.5 ± 1.0	***
	BA (cm^3)	24.6 ± 1.5	12.5 ± 1.3	***
	Bone diameter (cm)	6.6 ± 0.2	4.9 ± 0.2	***
	Bone radius (cm)	3.3 ± 0.1	2.5 ± 0.1	***
	Marrow radius (cm)	1.7 ± 0.1	1.4 ± 0.1	***
	Wall thickness (cm)	1.6 ± 0.1	1.0 ± 0.1	***
	CSA: MA	3.7 ± 0.5	3.0 ± 0.3	***
	BA %	72.5 ± 3.0	66.0 ± 3.6	**
Mc	CSA (cm^2)	29.0 ± 1.7	12.2 ± 1.8	***
	MA (cm^3)	6.3 ± 1.6	3.3 ± 0.8	***
	BA (cm^3)	22.7 ± 2.9	8.8 ± 1.4	***
	Bone diameter (cm)	6.1 ± 0.2	3.9 ± 0.3	***
	Bone radius (cm)	3.0 ± 0.1	2.0 ± 0.1	***
	Marrow radius (cm)	1.4 ± 0.1	1.0 ± 0.1	***
	Wall thickness (cm)	1.6 ± 0.2	0.9 ± 0.1	***
	CSA: MA	4.9 ± 1.4	3.8 ± 0.8	*Ns*
	BA %	78.4 ± 6.4	72.7 ± 5.2	*Ns*

ns Not significant * P<0.05 ** P<0.01 *** P<0.001

When these results are compared between *Fs* and *Mc* within a species, it shows interesting differences (*Table 3.13*).

Table 3.13: *Comparison of intraspecific differences in bone morphology of giraffes and buffaloes*

		Fs	***Mc***	
	Measurement	**mean±SD**	**mean±SD**	**Significance**
Giraffe	CSA (cm^2)	33.9 ± 1.6	29.0 ± 1.7	***
	MA (cm^3)	9.3 ± 1.1	6.3 ± 1.6	**
	BA (cm^3)	24.6 ± 1.5	22.7 ± 2.9	*ns*
	Bone diameter (cm)	6.6 ± 0.2	6.1 ± 0.2	*ns*
	Bone radius (cm)	3.3 ± 0.1	3.0 ± 0.1	***
	Marrow radius (cm)	1.7 ± 0.1	1.4 ± 0.1	*ns*
	Wall thickness (cm)	1.6 ± 0.1	1.6 ± 0.2	*ns*
	CSA: MA	3.7 ± 0.5	4.9 ± 1.4	*ns*
	BA %	72.5 ± 3.0	78.4 ± 6.4	*ns*
Buffalo	CSA (cm^2)	19.0 ± 1.8	12.2 ± 1.8	***
	MA (cm^3)	6.5 ± 1.0	3.3 ± 0.8	***
	BA (cm^3)	12.5 ± 1.3	8.8 ± 1.4	***
	Bone diameter (cm)	4.9 ± 0.2	3.9 ± 0.3	***
	Bone radius (cm)	2.5 ± 0.1	2.0 ± 0.1	***
	Marrow radius (cm)	1.4 ± 0.1	1.0 ± 0.1	***
	Wall thickness (cm)	1.0 ± 0.1	0.9 ± 0.1	*ns*
	CSA: MA	3.0 ± 0.3	3.8 ± 0.8	*
	BA %	66.0 ± 3.6	72.7 ± 5.2	**

ns Not significant * P<0.05 ** P<0.01 *** P<0.001

CSA, MA and BA differed significantly (P<0.001) interspecifically. When the ratio of *Fs*:*Mc* CSA was calculated it was found to be 1.17 in giraffes and 1.58 in buffaloes, which are significantly different (P<0.001) and emphasize that giraffes have a relatively greater *Mc* CSA than do buffalo.

BA in giraffe *Fs* and *Mc* were not significantly different (P>0.05) while in buffaloes the *Fs* had a significantly (P<0.001) greater BA than the *Mc*, again showing a relatively high *Mc* BA in giraffes.

BA % was greater (P=0.0035) in giraffe *Fs* than that of buffaloes, while it was statistically the same (P>0.05) for *Mc*. In giraffes the *Fs* BA % did not differ significantly (P>0.05) from that of the *Mc*. However, this was not the case in buffaloes, where the *Fs* BA % was significantly (P=0.0072) less than that of the *Mc*. When BA %:D for *Fs* and *Mc* was calculated, it showed an apparently even greater (P<0.001) BA % in the *Mc* of buffaloes compared to *Fs,* whereas in giraffes this ratio didn't show any differences (P>0.05). Therefore it can be deduced that, even though the BA in the buffalo *Mc* was much less than that of the *Fs*, the BA % of the buffalo *Mc* was relatively much greater compared to that of the giraffe. This is concluded despite the fact that BA % did not directly differ between *Mc* of both species. This is paradoxical to the finding in absolute BA. Of the two, absolute bone area would be the more significant finding, since these two species fall within the same body mass category.

Outer (total bone radius) and inner (marrow radius) radius, calculated from the surface area results using the simple formula: $A = \pi r^2$ and wall thickness are also listed in *Table 3.12 & 3.13* above. *Fig 3.6* depicts these results assuming the bones to be cylindrical.

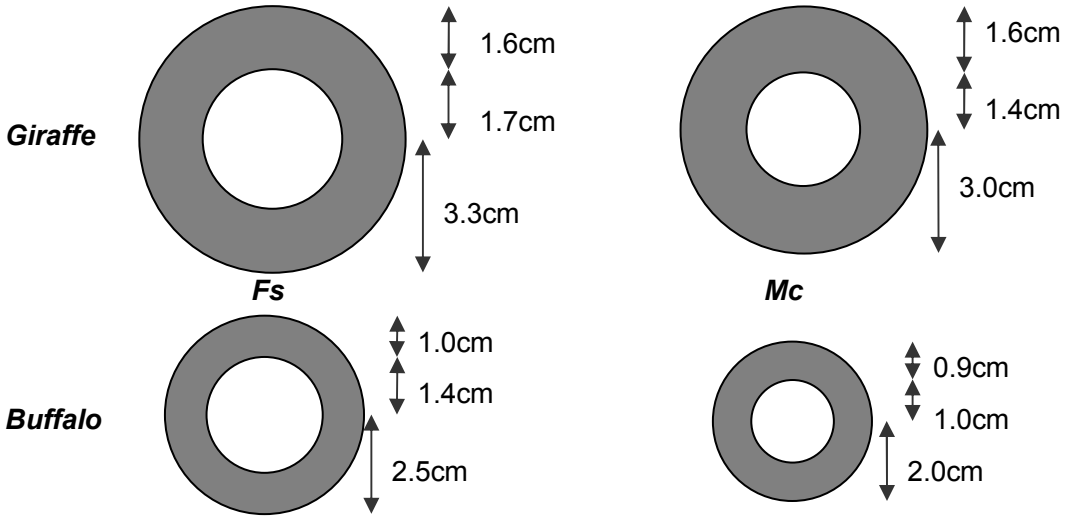

Fig 3.6: *Graphic representation (cylindrical) of bone cross-sectional areas*

The ratios of bone marrow diameter to outside diameter (*k*), for the results from *Table 3.13* are listed in *Table 3.14*. These *k* values are used to quantify economy of use of bone material for derived strength.

Table 3.14: *Bone marrow diameter:Outside diameter (k) for Fs and Mc*

	Giraffe			Buffalo		
	Marrow diameter	Outside diameter	*k*	Marrow diameter	Outside diameter	*k*
Fs	3.4	6.6	0.52	2.8	4.9	0.57
Mc	2.8	6.1	0.46	2.0	3.9	0.51

While the femur cross-sectional shape was close to cylindrical (*Fig 3.7*), the metacarpal shape, while anteriorly cylindrical, had a posterior concavity which was more marked in giraffes than in buffaloes (*Fig 3.8*). *Fig 3.7 & 3.8* also show the marked differences in wall thickness, both inter- and intraspecifically.

Fig 3.7: *Photograph showing geometrical shape of all femur cross sections. The top two rows are giraffe femurs while the bottom two rows are those of buffaloes*

Fig 3.8: *Photograph showing geometrical shape of all metacarpus cross sections. The top two rows are giraffe metacarpi while the bottom three rows are those of buffaloes*

CHAPTER IV

DISCUSSION

The aim of this study was to establish if giraffes had any unique features regarding their bone density, skeletal calcium (Ca) and phosphorus (P) content and certain femoral and metacarpal morphological characteristics. These were investigated because of the uniqueness of the giraffe's vertical growth rate, skeletal biomechanics, dietary mineral balance and relatively large skeletal mass. The African buffalo served as the "conventional control" against which these features were measured.

The findings of the present study do not indicate any one specific adaptation in the giraffe skeleton, but rather at various seemingly smaller adaptations that, through intricate interplay, may have made extant *G. camelopardalis* successful enough to out compete all of its relatives.

When compared to the buffalo, the elongated neck and legs of the giraffe are the distinguishing characteristics in their skeletal shape. Any biomechanical adaptations relating to bone and mineral density would therefore be expected in these areas. On the other hand, any physiological adaptations relating to the same factors due to a seemingly challenging dietary mineral content and increased vertical growth rate would be expected in the areas that are able to afford a decrease in bone and or mineral density and thus strength. This would include bones like the ribs, tuber coxae, vertebrae, etc.

4.1 BONE DENSITY

Mitchell & Skinner (2003a) recently proposed a lower bone density in the giraffe cervical vertebrae and denser metapodials compared to other mammals. The results of the present study do not support their hypothesis, since no difference in bone density between similar bones and especially the cervical vertebrae or metapodial bones of the giraffe and buffalo could be found. The only statistically significant difference was found in the grouped results of the lumbar vertebrae, which on its own has little biological significance.

Therefore, one could say that the rapid vertical growth, seemingly adverse dietary mineral balance and skeletal shape have no influence on bone density of adult giraffe bulls.

Bone density definitely appears to be an important factor in bone strength, as could be seen in the intraspecific comparisons between bone samples. Bones supporting body mass consistently had a significantly higher density than non-weight bearing bones (e.g. metacarpi *cf.* vertebrae). The cross-sectional area of the bone area upon which the main weight bearing force acted, also seemed to be inversely related to its density. This could be seen in the substantial difference in bone density between femoral diaphyses and epiphyses and the lower density of carpals compared to long bone shafts. These findings confirm those of Brain (1981, pers comm.) and Benzie *et al*. (1955; 1959) on bone density and those of Pauwels (1980) on

epiphyseal diameter compared to diaphyseal diameter in relation to density. Furthermore, femur heads had a bone density similar to vertebrae and this is most probably due to their similar cancellous structure, which is well-known to facilitate remodelling.

The relationship between bone density and function was also clearly shown in the grouping of bones according to density with the long bone shafts being densest, followed by carpals, then ribs, vertebrae and femur heads and the least dense tuber coxae.

The results of the present study show that the absolute mass of giraffe cervical vertebrae is significantly higher than that of buffaloes while the difference in mass of the lumbar vertebrae is much less between the two species. The high giraffe cervical mass is a result of course of their elongation (they have a volume 4-5 fold that of buffalo vertebrae) and occurs despite an absence of bony insertion sites for muscles and ligaments (Lankester, 1908; Solounias, 1999). This result is paradoxical. It seems that a prerequisite for neck elongation should be a relative reduction in neck mass. If gaining access to nutrients is one evolutionary pressure for neck elongation in giraffes (albeit highly unlikely – see Mitchell & Skinner, 2003a), then a light and manoeuvrable neck might have utility. A heavy neck with bones of equal density but greater mass than that of buffaloes suggests that, discounting access to nutrients as a prime purpose of the neck, the giraffe neck has to withstand significant forces such as those that might

be expected during “necking” behaviour (Coe, 1967), and/or that it serves to counterbalance the body during galloping (Dagg & Foster, 1976). On the other hand, as *Fig 3.5* shows, giraffe cervical vertebrae mass appears to decrease from proximal to distal while that of buffalo vertebrae does not. The decrease in mass (and volume, as density stays constant) with increase in cranial distance in the giraffe means first, that the mass of its head and neck is supported mainly at the base and secondly that the cranial extremity will be comparatively light and more manoeuvrable than if there was no gradation as is the case in buffaloes. Although only three of the seven cervical vertebrae were analysed in this study, my observation was that the other four cervical vertebrae (C1, C2, C6 and C7) also conformed to this trend. Thus, the differential densities and other morphological features found could have biological significance, and may be economical of calcium and phosphate acquisition.

4.2 MINERAL CONTENT

The results of this part of the study show that the giraffe skeleton contains more absolute amounts of Ca and P than is found in the buffalo skeleton, although the concentration of Ca and P in the bones of both species is similar. These findings therefore provide little support for the idea that giraffes are in critical Ca balance. However, some evidence indicates that the Ca concentration of giraffe bones, like that of serum Ca concentration, is more variable than the P concentration. Given that the Ca content of giraffe browse is several fold higher than it is in buffalo graze, the high variability is surprising. It might be expected that the Ca concentration of buffalo bones would vary more. A possibility is that the giraffe intestinal tract is unable to balance calcium and phosphate absorption. However, there is no good information published on the bio-availability of minerals in the giraffe's gastro-intestinal tract.

Another possible reason for the variation, at least in giraffes, is that they have to accumulate 1.5 to 2.0 times as much Ca during growth to maturity as do buffaloes, and it is reasonable to conclude that Ca and P deficiency is therefore more likely to occur in them than, say, in buffaloes. Assuming that the two species reach maturity at a similar age, that skeletal mass of giraffes is greater, and that daily food consumption is similar at about 2.2-2.4% of body mass dry matter intake (Dagg & Foster, 1976; Du Toit, 2000), it is impossible for them to meet their estimated needs of 20g per day of calcium (at 100%

absorption, Mitchell & Skinner, 2003b) from grass. Grass has a Ca:P ratio of 2:1 and a calcium content nearly five times less (eight g per 20kg of dry matter) than is found in giraffe browse (40 g per 20kg dry matter) (Dougall *et al*., 1964). Giraffes are, it seems, obliged to eat dicotyledenous browse with a high Ca content in order to obtain sufficient Ca for their skeletal growth. Small changes in availability of Ca in browse could then explain the variations in Ca content of giraffe bones, and a calcium deficiency must result if they do not have access to Ca-rich food. Their extinctions in Asia and China can be explained by these conclusions. The variations in buffalo skeletal Ca concentration can be attributed to the lower Ca content of grass.

However, these results also suggest that extant African giraffes generally are able to obtain adequate Ca. On the other hand, the origin of sufficient P is less apparent. Giraffe and buffalo bone P concentration (g/g) is on average identical, as is the P content of their diets (0.2%) (Dougall *et al*., 1964), yet giraffes must accumulate 1.5 to 2 times as much P than buffaloes do to have a Ca:P ratio in their bones similar to that in buffalo (about 2.1:1). The range of serum P concentration reported for giraffes is wider than it is in buffaloes and is generally more variable than is serum Ca concentration. Therefore, the frequent observations of osteophagia reported in giraffes may perhaps be a sign not of Ca deficiency, but rather of P deficiency, as it is in cattle *Bos taurus* (Theiler *et al*., 1924; McDowell *et al*., 1992). However, values for *Rib* P density (g/cm^3) in this study correlated well

with those of other workers, mainly studying P deficiency in cattle and sheep *Ovis aries* (Little, 1972; Read *et al*., 1986; Williams *et al*., 1990). According to these studies a P density of 0.12g/cm^3 and lower indicates a P deficiency, while 0.14-0.15g/cm^3 indicates adequate intake. Both the giraffe and buffalo rib P density in this study fell above the deficiency threshold (*Table 4.1*). Although no standard for giraffe rib biopsies has been set, my data does not support the idea that adult giraffe bulls suffer from any form of chronic P deficiency. In addition, in other animals suffering from a Ca and P deficiency, demineralization of bone and a reduction in bone mass occurs, and, therefore, both a reduction in bone density and bone mineral density is seen (Shupe *et al*., 1988). These results can show neither in adult giraffe bulls.

Table 4.1: *Rib P density (g/cm^3) values of each carcass*

Animal #	Giraffe	Buffalo
1	0.13	
2	0.14	0.13
3	0.13	0.15
4	0.15	0.15
5	0.15	0.17
6	0.15	0.15
7		0.18
8		0.15
9		0.15
Mean	**0.142**	**0.154**
	±0.01	**±0.02**

The most comprehensive analysis of plants eaten by giraffe and other African herbivores is that of Dougall *et al.* (1964). They identified 137 non-graminaceous plants eaten by various herbivores, and collected and analysed these plants for, amongst other things, Ca and P content.

A summary of their findings is shown in *Tables 4.2(a)* and *(b)*, in which giraffe diets are compared with those of eland *Tragelaphus oryx* (a putative competitor), bovids, and antelope. These data indicate that giraffe browse contains higher concentrations of Ca and that competition for this browse is minimal. There is overlap of browse species eaten, but the overlapping species do not contain high Ca. The data also show that the P content of giraffe browse is significantly lower in some cases which further emphasizes the paradox of P balance in giraffes.

Table 4.2(a): *Ca and P content of browse (derived from Dougall et al., 1964)*

Animal Species	Browse Species• (N)	[Ca]* (%)	[P]* (%)	Ca:P	Overlap with Giraffe#
Eland	3	1.0 ± 0.4	0.4 ± 0.2	3.5 ± 2.2	1/3
Ruminants	45	1.3 ± 1.0	0.3 ± 0.1	3.5 ± 1.8	6/45
Antelope	13	1.3 ± 0.7	0.3 ± 0.1	4.6 ± 2.4	3/13
a) Giraffe	13	1.7 ± 2.8	0.2 ± 0.1	7.7 ± 2.6	-
b) Giraffe	4	2.6 ± 0.6	0.2 ± 0.04	18.4 ± 5.1	-
Giraffe a+b	17	1.9 ± 2.3	0.2 ± 0.07	8.2 ± 3.2	-

• = Number of browse species eaten by each of the groups of animal

* = Ca and P concentration in the browse species eaten

= Number of browse species eaten by giraffe and each of the other animal groups

Note that giraffe ate 4 species (*Cordia gharaf; Grewia similes; Acacia tortilis; Cadaba farinose*) with very high Ca content. The Ca content of the 17 species eaten by giraffes was significantly higher than that of eland browse, but not of antelope and bovid browse. The P content of eland browse was higher than giraffe browse. The Ca:P ratio in giraffe browse was higher than the ratio in the browse of the three other groups.

Table 4.2(b): *Ca and P content of Mimosaceae*

Animal Species	Browse Species (N)	[Ca] (%)	[P] (%)	Ca:P	Overlap with Giraffe
Eland	0	-	-	-	-
Ruminants	9	0.9 ± 0.5	0.3 ± 0.1	3.2 ± 2.0	3/9
Antelope	5	1.1 ± 0.6	0.2 ± 0.07	4.8 ± 3.5	3/5
Giraffe	6	1.1 ± 0.2	0.2 ± 0.07	5.3 ± 1.4*	-

* = The Ca:P ratio in giraffe Mimosaceae browse is significantly higher than it is in ruminant browse, but not different to that in antelope browse. Of the 17 Mimosaceae species eaten by ruminants, antelope and giraffe, 3 were eaten by giraffe alone (*Acacia dreparolibum; Acacia stuhlmannii; Dichrostachys cinerea*).

The significantly lower mineral concentration of *C5* and *L3* in giraffes compared to buffaloes implies that these bones should be weaker than those in the buffalo. Bone quality (i.e. breaking strength) is related to mineral content as a percentage of bone mass (Gaynor Evans, 1973). However, with no difference in the density of the same bone between the two species, strength may depend on what is used as 'replacement material' for the lack of minerals, and the nature of the microstructure of the bones (Gaynor Evans, 1973). Strength could even be unaffected or increased. However, in the six skeletons collected, two cases of possible non-union in fractured giraffe cervical spinous processes were observed. Although this could be a normal phenomenon due to violent 'necking' behaviour, it could also indicate a weaker bone structure and a delay in healing time due to a low mineral content.

4.3 CROSS-SECTIONAL AREA

Increased long bone strength in giraffes, according to the results of the present study, seems to be derived rather from an increase in bone diameter and bone wall thickness than increased mineral or bone density.

Alexander (1977; 1979) & Biewener (1983) both showed that bone diameter increases proportionally to an increase in bone length. This study showed a 1-2cm greater diameter in giraffe metacarpi and femurs compared to buffaloes. Although significant, this is not reflected in the much greater difference in increase in length of especially the giraffe's metapodials compared to the buffalo's.

The increase in wall thickness of the giraffe long bones, on the other hand, is much greater. Giraffe femurs had a 0.8cm greater diameter than buffaloes, and of this 0.6cm is an increase in wall thickness. In the metacarpus the same applies with the giraffe having a 1cm greater metacarpal diameter of which again 0.6cm is an increase in wall thickness. Moreover, the giraffe metacarpal cross-sectional shape appeared to have two 'columns' caudally, forming two imaginary pillars, which could be an adaptive mechanism for increasing strength in these slender bones (*Fig 4.1*). Due to the elongation of giraffe bones, even though having a smaller diameter, their marrow cavities are not expected to have a decreased volume.

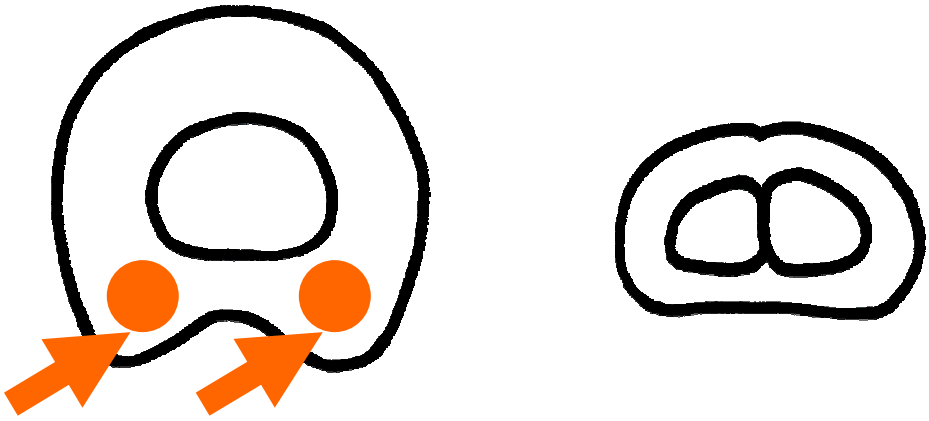

Fig 4.1: *Giraffe (left) and buffalo (right) metacarpal cross-sectional shapes showing the two areas of caudal thickening in the giraffe metacarpus (arrows).*

An increase in wall thickness definitely increases bone strength, although to a relatively lesser extent (Pauwels, 1980; Alexander, 1982). In the present study the lengths of bones were not measured and therefore the ultimate effect of these thicker walls could not be quantified but, as a sole adaptation to increase bone strength/decrease loading force, it would, in my opinion, not be adequate except if seen in combination with other biomechanical adaptations.

Alexander (1982) and Pauwels (1980) both calculated the optimal marrow cavity diameter to outside diameter ratio (*k*) to be in the region of 0.63 (ranging from 0.4-0.7). In Alexander's work, *k* for buffalo femurs was found to be 0.54 compared to 0.57 found in the present study (*Table 3.14*). Giraffe femurs had a *k* value of 0.52. Metacarpal values were 0.51 and 0.46 for buffaloes and giraffes respectively. All values are therefore within the optimal range for bone material economy (Pauwels, 1980).

Although not measured directly, by observation alone and deduction from data of previous workers (Gambaryan, 1974), it was clear that the angle of both the neck and metacarpals of the giraffe were much closer to the vertical than it was in buffaloes. Such a postural change causes a significant decrease in horizontal loading forces and would therefore decrease the demand for increasing strength in these elongated features of the giraffe. *Fig 4.2* illustrates this observation.

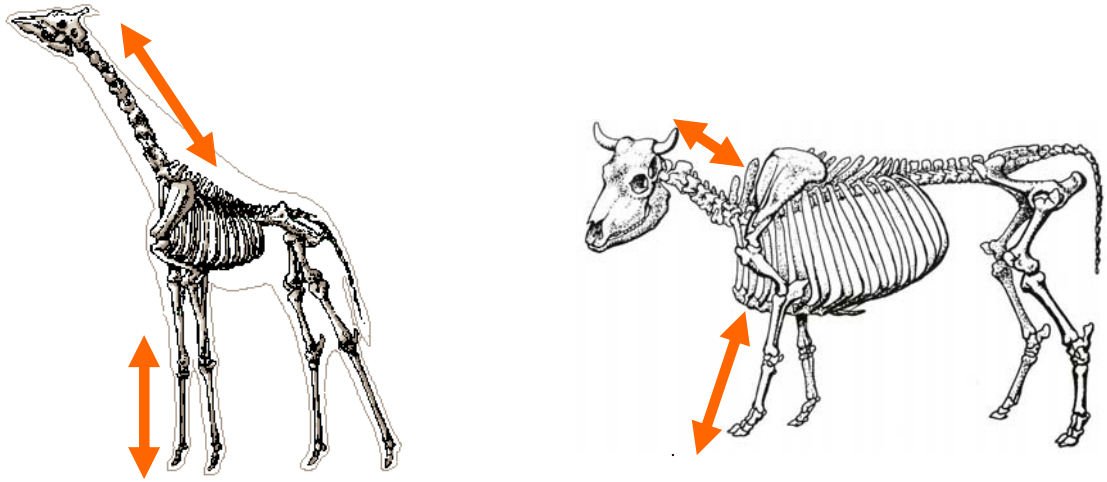

Fig 4.2: *Giraffe (left) and bovid (right) skeletons, illustrating the different angles of the neck and metacarpals with relation to the vertical (arrows).*

4.4 CONCLUSIONS

The results of the present study indicate that, despite the unique anatomy of the giraffe, its high vertical growth rate, the high proportion of its body mass that is skeleton, and exposure to an adverse dietary Ca:P ratio, its skeleton is not unique at least with respect to bone and mineral density.

Bone density seems to have function in bone strength, as is reflected in the similar intraspecific differences between giraffes and buffaloes. This attribute seems not to be affected by gross anatomy and or skeletal shape. The tapering mass of the giraffe cervical vertebrae, decreased vertical angle of the neck, decreased skull weight and decreased muscle mass serves to increase manoeuvrability and facilitate carriage of the giraffe neck.

Moreover, a combination of increased wall thickness, apparent reinforced cross-sectional shape, increased long bone density and decreased vertical angle of the giraffe metacarpus, seems to act as possible adaptations to the biomechanical demands imposed upon it by its slender shape.

In summary the mineral results have shown that the density of bones is correlated significantly but not completely with their Ca and P content. While significant differences can be shown in Ca and P content between giraffe and buffalo bones, actual differences are small. More

importantly, these results show that the absolute amounts of Ca and P that have to be accumulated by giraffes during their rapid growth period is higher than it is in buffaloes. The Ca appears to be acquired by selection for high Ca dicotyledenous browse (Dougall *et al*., 1964). Acquisition of the necessary required P on the other hand is revealed as a deficiency that is more likely to occur and may explain giraffe osteophagia, contrary to the expectations of Mitchell & Skinner (2003a).

It would therefore seem that there is evidence of adaptations by the giraffe to its unique anatomy, growth rate and diet. Although these adaptations seem relatively small, their synergism is powerful, as is reflected by the success of the giraffe as a species.

4.5 FUTURE WORK

This project has provided valuable baseline data on giraffe and buffalo bone density and skeletal mineral content. However, it has also raised many questions. The following aspects regarding the giraffe would require further investigation:

- Diet
    - Mineral absorption studies in giraffes to determine their ability to absorb Ca and P and whether the high Ca:P of their diet plays a role in its absorption, as it does in other ruminants.
    - Establishment of effective and practical ways of determining mineral status in live giraffes and determination of normal ranges.
    - It has to be established whether osteophagia is just a behavioural reaction to Ca and/or P deficiency or whether it acts as a mechanism in alleviating an adverse dietary mineral balance
    - With regard to the previous point: the site of bone digestion after ingestion (i.e. rumen/abomasum) should be determined and also the effectiveness of this digestion
    - The effect of mineral (especially P) supplementation on the occurrence of botulism should be investigated as a possible health management tool in the game farming industry

- The effect of dietary mineral balance in lactating cows and growing calves should be investigated to determine how these animals survive under increased physiological (mineral) stress. (Practicality [?])

- Biomechanics
  - Limbs
    - Investigations into limb bones, especially metapodials, regarding their length, cross-sectional area, diameter, curvature, vertical angle and geometrical shape to quantify what the effect of these parameters are on biomechanical demand of bones, and thus how the slender bones of the giraffe ultimately sustain its loading forces

  - Neck
    - All seven cervical vertebrae need to be analysed to determine if the findings of this study applies throughout this part of the vertebral column.
    - Vertebral strength should be compared to other species to see whether the decreased mineral concentration of giraffe vertebrae has a detrimental effect on its strength.

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APPENDIX A

RAW DATA

Sample Nr	Bone Sample	Species	Fresh Weight g	Fresh Volume cm^3	Density g/cm^3	Relative Density %	Ca %	P %	Bone cross-sectional area cm^2	Marrow cross-sectional area cm^2
Gc0101	*C3*	Giraffe	1504	1074.9	1.4	72.80	19.45	9.18		
Gc0102	*C4*	Giraffe	2014	1525.4	1.3	68.70	18.65	9.03		
Gc0103	*C5*	Giraffe	2181	1863.1	1.2	60.91	17.70	8.59		
Gc0104	*L2*	Giraffe	479	357.2	1.3	69.77				
Gc0105	*L3*	Giraffe	454	333.0	1.4	70.94	19.49	9.46		
Gc0106	*Fp*	Giraffe	1063	908.4	1.2	60.89	21.68	9.72		
Gc0107	*Fs*	Giraffe	541	281.5	1.9	100.00	22.80	11.20	32.56	7.20
Gc0108	*Fd*	Giraffe	1843	1689.1	1.1	56.78				
Gc0109	*rC*	Giraffe	137	95.9	1.4	74.31	19.99	9.10		
Gc0110	*iC*	Giraffe	127	78.4	1.6	84.33				
Gc0111	*uC*	Giraffe	127	72.8	1.7	90.79				
Gc0112	*Mc*	Giraffe	609	344.1	1.8	92.10	23.18	11.37	26.94	6.21
Gc0113	*Rib*	Giraffe	128	99.8	1.3	66.75	19.60	9.75		
Gc0114	*Tc*	Giraffe	152	147.7	1.0	53.54				
Gc0301	*C3*	Giraffe	1608	1267.4	1.3	74.05	17.84	9.24		
Gc0302	*C4*	Giraffe	2007	1705.9	1.2	68.67	17.45	8.90		
Gc0303	*C5*	Giraffe	2497	2082.0	1.2	70.00	15.14	8.07		
Gc0304	*L2*	Giraffe	496	403.4	1.2	71.76				
Gc0305	*L3*	Giraffe	554	431.6	1.3	74.92	16.75	8.93		
Gc0306	*Fp*	Giraffe	1224	936.4	1.3	76.29	19.82	9.42		
Gc0307	*Fs*	Giraffe	526	307.0	1.7	100.00	21.33	10.81	34.45	10.49
Gc0308	*Fd*	Giraffe	2805	2262.3	1.2	72.37				
Gc0309	*rC*	Giraffe	177	145.3	1.2	71.11	18.53	9.30		
Gc0310	*iC*	Giraffe	151	118.0	1.3	74.69				
Gc0311	*uC*	Giraffe	146	105.7	1.4	80.61				
Gc0312	*Mc*	Giraffe	662	361.4	1.8	106.90	21.18	10.89	30.54	6.85
Gc0313	*Rib*	Giraffe	160	114.5	1.4	81.56	18.78	9.75		
Gc0314	*Tc*	Giraffe	270	245.9	1.1	64.08	18.32	8.40		

Sample Nr	*Bone Sample*	*Species*	*Fresh Weight*	*Fresh Volume*	*Density*	*Relative Density*	*Ca*	*P*	*Bone cross-sectional area*	*Marrow cross-sectional area*
			g	cm^3	g/cm^3	%	%	%	cm^2	cm^2
Gc0401	*C3*	Giraffe	1960	1357.6	1.4	75.21	18.91	8.26		
Gc0402	*C4*	Giraffe	2213	1589.9	1.4	72.52	19.54	9.52		
Gc0403	*C5*	Giraffe	2491	1890.9	1.3	68.63	18.01	8.74		
Gc0404	*L2*	Giraffe	458	354.4	1.3	67.33				
Gc0405	*L3*	Giraffe	514	399.2	1.3	67.08	18.33	9.06		
Gc0406	*Fp*	Giraffe	1291	1008.9	1.3	66.66	20.41	9.34		
Gc0407	*Fs*	Giraffe	535	278.7	1.9	100.00	21.65	10.72	34.51	9.36
Gc0409	*rC*	Giraffe	166	107.9	1.5	80.13	19.81	9.75		
Gc0410	*iC*	Giraffe	144	96.5	1.5	77.74				
Gc0411	*uC*	Giraffe	138	91.1	1.5	78.94				
Gc0412	*Mc*	Giraffe	705	358.7	2.0	102.39	21.45	10.48	28.94	6.40
Gc0413	*Rib*	Giraffe	80	58.3	1.4	71.50	18.53	9.50		
Gc0414	*Tc*	Giraffe	343	278.1	1.2	64.24	17.74	8.58		
Gc0501	*C3*	Giraffe	1555	1138.6	1.4	70.16	16.48	7.72		
Gc0502	*C4*	Giraffe	1881	1442.6	1.3	66.98	18.46	9.00		
Gc0503	*C5*	Giraffe	2204	1638.4	1.3	69.11	18.27	8.99		
Gc0504	*L2*	Giraffe	489	363.4	1.3	69.14				
Gc0505	*L3*	Giraffe	468	333.9	1.4	72.00	18.11	8.86		
Gc0506	*Fp*	Giraffe	1112	805.6	1.4	70.91	20.76	9.78		
Gc0507	*Fs*	Giraffe	395	202.9	1.9	100.00	21.13	10.09	31.32	9.38
Gc0508	*Fd*	Giraffe	2234	1694.4	1.3	67.73				
Gc0509	*rC*	Giraffe	159	102.4	1.6	79.75	17.93	8.25		
Gc0510	*iC*	Giraffe	147	95.1	1.5	79.44				
Gc0511	*uC*	Giraffe	127	80.0	1.6	81.56				
Gc0512	*Mc*	Giraffe	414	221.3	1.9	96.12	23.43	11.00	26.96	8.81
Gc0513	*Rib*	Giraffe	63	39.0	1.6	82.99	20.90	9.37		
Gc0514	*Tc*	Giraffe	133	105.1	1.3	65.03	17.83	8.47		

Sample Nr	*Bone Sample*	*Species*	*Fresh Weight*	*Fresh Volume*	*Density*	*Relative Density*	*Ca*	*P*	*Bone cross-sectional area*	*Marrow cross-sectional area*
			g	cm^3	g/cm^3	%	%	%	cm^2	cm^2
Gc0601	*C3*	Giraffe	2135	1563.4	1.4	69.93	16.46	8.51		
Gc0602	*C4*	Giraffe	2609	1955.1	1.3	68.34	18.41	8.99		
Gc0603	*C5*	Giraffe	3155	2385.6	1.3	67.72	21.50	9.69		
Gc0604	*L2*	Giraffe	544	402.2	1.4	69.26				
Gc0605	*L3*	Giraffe	587	422.4	1.4	71.17	18.47	9.01		
Gc0606	*Fp*	Giraffe	1463	1126.1	1.3	66.53	19.90	10.02		
Gc0607	*Fs*	Giraffe	490	250.9	2.0	100.00	18.97	9.01	35.66	9.47
Gc0609	*rC*	Giraffe	174	109.1	1.6	81.69		9.89		
Gc0610	*iC*	Giraffe	163	100.4	1.6	83.12				
Gc0611	*uC*	Giraffe	171	96.5	1.8	90.75				
Gc0612	*Mc*	Giraffe	585	277.6	2.1	107.93	20.55	10.86	29.85	4.33
Gc0613	*Rib*	Giraffe	154	96.8	1.6	81.48	21.10	9.58		
Gc0614	*Tc*	Giraffe	146	110.0	1.3	67.97	20.18	9.24		
Gc0701	*C3*	Giraffe	2012	1446.4	1.4	67.93	20.22	9.53		
Gc0702	*C4*	Giraffe	2566	1902.3	1.3	65.87	19.03	8.59		
Gc0703	*C5*	Giraffe	2978	2206.1	1.3	65.92	18.18	8.74		
Gc0704	*L2*	Giraffe	555	422.7	1.3	64.12				
Gc0705	*L3*	Giraffe	550	422.9	1.3	63.51	19.85	8.94		
Gc0706	*Fp*	Giraffe	1405	1056.9	1.3	64.92	22.41	10.40		
Gc0707	*Fs*	Giraffe	447	218.3	2.0	100.00	24.71	11.37	34.61	9.92
Gc0708	*Fd*	Giraffe	3356	2649.3	1.3	61.86				
Gc0709	*rC*	Giraffe	214	135.4	1.6	77.21	18.83	9.09		
Gc0710	*iC*	Giraffe	195	117.9	1.7	80.80				
Gc0711	*uC*	Giraffe	189	107.0	1.8	86.26				
Gc0712	*Mc*	Giraffe	438	222.8	2.0	96.01	20.34	10.47	30.84	5.02
Gc0713	*Rib*	Giraffe	116	75.5	1.5	75.03	21.32	9.64		
Gc0714	*Tc*	Giraffe	265	210.1	1.3	61.58	20.02	9.11		

Sample Nr	*Bone Sample*	*Species*	*Fresh Weight*	*Fresh Volume*	*Density*	*Relative Density*	*Ca*	*P*	*Bone cross-sectional area*	*Marrow cross-sectional area*
			g	cm^3	g/cm^3	%	%	%	cm^2	cm^2
Sc0101	*C3*	Buffalo	600	446.9	1.3			9.33		
Sc0102	*C4*	Buffalo	627	474.7	1.3		18.51	8.66		
Sc0103	*C5*	Buffalo	656	487.3	1.3		19.87	9.07		
Sc0109	*rC*	Buffalo	52	36.9	1.4		19.99	9.75		
Sc0110	*iC*	Buffalo	41	31.5	1.3					
Sc0111	*uC*	Buffalo	42	30.1	1.4					
Sc0112	*Mc*	Buffalo	130	75.1	1.7		22.19	10.78	12.71	3.99
Sc0113	*Rib 1*	Buffalo	65	47.4	1.4					
Sc0201	*C3*	Buffalo	530	382.6	1.4	74.72	18.97	8.79		
Sc0202	*C4*	Buffalo	624	441.9	1.4	76.17	18.80	9.11		
Sc0203	*C5*	Buffalo	608	429.3	1.4	76.40	18.26	9.14		
Sc0204	*L2*	Buffalo	373	287.8	1.3	69.91				
Sc0205	*L3*	Buffalo	357	267.5	1.3	71.99	21.57	9.15		
Sc0206	*Fp*	Buffalo	858	669.2	1.3	69.16	17.73	8.81		
Sc0207	*Fs*	Buffalo	212	114.4	1.9	100.00	25.30	10.98	20.07	7.50
Sc0208	*Fd*	Buffalo	996	759.1	1.3	70.77				
Sc0209	*rC*	Buffalo	57	42.1	1.4	73.08	21.45	9.10		
Sc0210	*iC*	Buffalo	41	32.4	1.3	68.35				
Sc0211	*uC*	Buffalo	47	36.8	1.3	68.92				
Sc0212	*Mc*	Buffalo	100	59.1	1.7	91.21	24.08	10.29	12.75	4.00
Sc0213	*Rib*	Buffalo	54	39.6	1.4	73.48	21.76	9.64		
Sc0214	*Tc*	Buffalo	183	158.1	1.2	62.45	15.55	7.13		
Sc0304	*L2*	Buffalo	301	211.8	1.4	73.28				
Sc0305	*L3*	Buffalo	333	226.4	1.5	75.83	19.02	9.00		
Sc0306	*Fp*	Buffalo	695	543.8	1.3	65.90	19.13	9.15		
Sc0307	*Fs*	Buffalo	229	118.1	1.9	100.00			18.89	6.83
Sc0308	*Fd*	Buffalo	864	661.1	1.3	67.38				
Sc0309	*rC*	Buffalo	42	28.4	1.5	76.37	19.78	9.18		
Sc0310	*iC*	Buffalo	35	24.2	1.4	74.53				
Sc0311	*uC*	Buffalo	34	21.4	1.6	81.81				
Sc0312	*Mc*	Buffalo	95	53.5	1.8	91.55	22.48	10.30	12.40	3.88
Sc0313	*Rib*	Buffalo	59	39.4	1.5	77.29	20.49	9.71		
Sc0314	*Tc*	Buffalo	76	65.9	1.2	59.44	16.81	8.20		

Sample Nr	*Bone Sample*	*Species*	*Fresh Weight*	*Fresh Volume*	*Density*	*Relative Density*	*Ca*	*P*	*Bone cross-sectional area*	*Marrow cross-sectional area*
			g	cm^3	g/cm^3	%	%	%	cm^2	cm^2
Sc0401	*C3*	Buffalo	495	414.0	1.2	59.83	20.66	9.24		
Sc0402	*C4*	Buffalo	544	374.5	1.5	72.69	17.78	9.44		
Sc0403	*C5*	Buffalo	557	433.9	1.3	64.24	20.63	9.34		
Sc0404	*L2*	Buffalo	376	268.9	1.4	69.98				
Sc0405	*L3*	Buffalo	425	296.6	1.4	71.70	19.73	9.54		
Sc0406	*Fp*	Buffalo	550	413.8	1.3	66.52	17.86	9.69		
Sc0407	*Fs*	Buffalo	170	85.1	2.0	100.00	23.20	10.67	18.59	7.29
Sc0408	*Fd*	Buffalo	690	510.8	1.4	67.60				
Sc0409	*rC*	Buffalo	49	29.3	1.7	83.73	21.18	9.21		
Sc0410	*iC*	Buffalo	35	21.1	1.7	82.84				
Sc0411	*uC*	Buffalo	45	26.7	1.7	84.30				
Sc0412	*Mc*	Buffalo	99	50.0	2.0	99.08	22.23	9.93	12.00	3.87
Sc0413	*Rib*	Buffalo	49	30.6	1.6	80.02	20.54	9.69		
Sc0414	*Tc*	Buffalo	185	156.4	1.2	59.18	18.91	8.42		
Sc0501	*C3*	Buffalo	461	294.8	1.6	77.53	18.13	9.16		
Sc0502	*C4*	Buffalo	491	323.1	1.5	75.33	20.04	9.96		
Sc0503	*C5*	Buffalo	500	321.4	1.6	77.12		9.84		
Sc0504	*L2*	Buffalo	361	262.2	1.4	68.25				
Sc0505	*L3*	Buffalo	366	259.7	1.4	69.86	18.85	8.50		
Sc0506	*Fp*	Buffalo	611	472.4	1.3	64.12	21.21	10.15		
Sc0507	*Fs*	Buffalo	218	108.1	2.0	100.00	22.13	11.27	17.50	5.29
Sc0508	*Fd*	Buffalo	754	591.6	1.3	63.19				
Sc0509	*rC*	Buffalo	59	33.2	1.8	88.06	20.12	9.96		
Sc0510	*iC*	Buffalo	34	19.7	1.7	85.50				
Sc0511	*uC*	Buffalo	45	22.1	2.0	101.07				
Sc0512	*Mc*	Buffalo	103	51.5	2.0	99.15	20.92	9.93	11.14	2.42
Sc0513	*Rib*	Buffalo	55	33.4	1.6	81.74	20.00	10.13		
Sc0514	*Tc*	Buffalo	98	94.7	1.0	51.29	18.43	9.47		

Sample Nr	*Bone Sample*	*Species*	*Fresh Weight*	*Fresh Volume*	*Density*	*Relative Density*	*Ca*	*P*	*Bone cross-sectional area*	*Marrow cross-sectional area*
			g	cm^3	g/cm^3	%	%	%	cm^2	cm^2
Sc0601	*C3*	Buffalo	566	388.2	1.5	76.07	19.21	9.62		
Sc0602	*C4*	Buffalo	610	423.6	1.4	75.13	20.13	8.81		
Sc0603	*C5*	Buffalo	622	444.6	1.4	73.00	19.80	9.07		
Sc0604	*L2*	Buffalo	329	233.1	1.4	73.63				
Sc0605	*L3*	Buffalo	355	249.6	1.4	74.22	22.20	9.70		
Sc0606	*Fp*	Buffalo	815	642.6	1.3	66.17	18.31	9.83		
Sc0607	*Fs*	Buffalo	187	97.6	1.9	100.00	22.72	10.96	19.24	6.56
Sc0608	*Fd*	Buffalo	881	684.8	1.3	67.13				
Sc0609	*rC*	Buffalo	53	35.2	1.5	78.53	18.22	9.67		
Sc0610	*iC*	Buffalo	40	28.1	1.4	74.16				
Sc0611	*uC*	Buffalo	43	29.9	1.4	75.15				
Sc0612	*Mc*	Buffalo	102	58.1	1.8	91.65	19.93	10.91	13.04	3.93
Sc0613	*Rib*	Buffalo	48	31.5	1.5	79.51	19.09	10.00		
Sc0614	*Tc*	Buffalo	229	206.4	1.1	57.90	15.56	8.35		
Sc0701	*C3*	Buffalo	568	363.7	1.6	79.93	18.13	6.87		
Sc0702	*C4*	Buffalo	635	414.3	1.5	78.45	18.83	7.33		
Sc0703	*C5*	Buffalo	636	417.9	1.5	77.89	18.60	9.86		
Sc0704	*L2*	Buffalo	393	270.9	1.5	74.27				
Sc0705	*L3*	Buffalo	428	286.4	1.5	76.50	20.11	9.46		
Sc0706	*Fp*	Buffalo	723	521.1	1.4	71.02	21.25	10.25		
Sc0707	*Fs*	Buffalo	235	120.3	2.0	100.00	22.86	10.98	18.56	5.29
Sc0708	*Fd*	Buffalo	837	620.6	1.3	69.04				
Sc0709	*rC*	Buffalo	51	33.4	1.5	78.09	17.90	9.18		
Sc0710	*iC*	Buffalo	36	23.9	1.5	77.24				
Sc0711	*uC*	Buffalo	41	25.5	1.6	82.30				
Sc0712	*Mc*	Buffalo	95	46.4	2.0	104.89	20.13	10.98	11.15	2.13
Sc0713	*Rib*	Buffalo	57	31.9	1.8	91.38	22.60	10.29		
Sc0714	*Tc*	Buffalo	105	90.5	1.2	59.39	19.85	9.24		

Sample Nr	*Bone Sample*	*Species*	*Fresh Weight*	*Fresh Volume*	*Density*	*Relative Density*	*Ca*	*P*	*Bone cross-sectional area*	*Marrow cross-sectional area*
			g	cm^3	g/cm^3	%	%	%	cm^2	cm^2
Sc0801	*C3*	Buffalo	300	247.9	1.2	60.69	18.31	8.77		
Sc0802	*C4*	Buffalo	304	272.9	1.1	55.88	20.90	9.43		
Sc0803	*C5*	Buffalo	304	273.6	1.1	55.73	18.59	9.06		
Sc0804	*L2*	Buffalo	254	199.4	1.3	63.88				
Sc0805	*L3*	Buffalo	270	209.9	1.3	64.53	19.96	9.93		
Sc0806	*Fp*	Buffalo	566	503.6	1.1	56.37	20.11	9.18		
Sc0807	*Fs*	Buffalo	141	70.7	2.0	100.00	22.68	11.13	16.46	5.56
Sc0808	*Fd*	Buffalo	627	523.7	1.2	60.04				
Sc0809	*rC*	Buffalo	41	27.6	1.5	74.58	19.04	9.34		
Sc0810	*iC*	Buffalo	32	18.1	1.8	88.81				
Sc0811	*uC*	Buffalo	35	21.1	1.7	83.02				
Sc0812	*Mc*	Buffalo	77	40.4	1.9	95.52	23.26	10.39	8.93	2.37
Sc0813	*Rib*	Buffalo	34	20.7	1.6	82.32	19.02	8.96		
Sc0814	*Tc*	Buffalo	126	117.4	1.1	53.85	17.87	7.93		
Sc0901	*C3*	Buffalo	659	488.6	1.3	68.58	22.93	10.06		
Sc0902	*C4*	Buffalo	650	496.0	1.3	66.64	21.33	9.36		
Sc0903	*C5*	Buffalo	655	519.1	1.3	64.16	22.20	10.19		
Sc0904	*L2*	Buffalo	371	274.5	1.4	68.72				
Sc0905	*L3*	Buffalo	460	350.1	1.3	66.80	21.74	9.60		
Sc0906	*Fp*	Buffalo	713	678.7	1.1	53.42	20.69	10.14		
Sc0907	*Fs*	Buffalo	202	102.7	2.0	100.00	24.30	10.95	22.33	7.36
Sc0908	*Fd*	Buffalo	844	771.6	1.1	55.62				
Sc0909	*rC*	Buffalo	64	39.5	1.6	82.39	20.40	9.50		
Sc0910	*iC*	Buffalo	37	22.3	1.7	84.42				
Sc0911	*uC*	Buffalo	46	26.7	1.7	87.56				
Sc0912	*Mc*	Buffalo	142	74.9	1.9	96.37	22.65	10.32	15.23	3.29
Sc0913	*Rib*	Buffalo	52	33.9	1.5	77.93	21.23	9.74		
Sc0914	*Tc*	Buffalo	146	181.4	0.8	40.94	18.13	8.45		