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COMPUTED TOMOGRAPHY FOR MEASURING BODY FAT RESERVES IN THE THREATENED MOJAVE DESERT TORTOISE (*GOPHERUS AGASSIZII*)

Margarete A. Walden, MS, Rachel Jania, DVM, Dipl ACVR, Matthew E. Kinney, DVM, Dipl ACZM, Anne Devan-Song, PhD, K. Kristina Drake, PhD, Todd C. Esque, PhD, and Kevin T. Shoemaker, PhD

Abstract: Noninvasive methods for measuring fat reserves in both captive and free-ranging animals are important for monitoring individual and population health, but chelonian anatomy and physiology present challenges to accurate measurements. Standard field-based methods for assessing body condition in Mojave desert tortoises (*Gopherus agassizii*) involve the qualitative body condition score, which relies on the apparent height of the temporalis muscle relative to the sagittal crest (in addition to other characteristics) and quantitative body condition indices that measure relative mass at size. However, it is unclear how these metrics relate to body fat reserves in this species. The aims of this study were to (1) describe the use of noninvasive computed tomography in measuring body fat volume of Mojave desert tortoises, (2) describe the location of fat reserves, (3) investigate relationships between fat reserves and body condition score and body condition index, and (4) explore whether relative temporalis muscle depth, measured via computed tomography, correlates with body condition score. Body condition scores were assessed for eight captive Mojave desert tortoises prior to euthanasia, and computed tomography was performed postmortem to quantify fat volume and measure temporalis muscle depth. At necropsy, the distribution of fat was documented. Fat volume calculated by computed tomography ranged from 2.83 to 145.38 cm^3 (0.07–2.5% body volume). Neither qualitative body condition score nor quantitative body condition index was correlated with fat volume. Bladder content did not compromise body condition index. Body condition score was not correlated with relative temporalis muscle depth. Computed tomography is a noninvasive method for successfully identifying fat reserves and estimating total fat volume in Mojave desert tortoises. The lack of a relationship between computed tomography-determined metrics and commonly used body condition metrics indicates that computed tomography fills a critical gap in the health assessment tool kit for captive and free-ranging Mojave desert tortoises.

INTRODUCTION

Fat in the form of triacylglycerol is the most energy-rich macromolecule used as an energy store for vertebrates.[45] Fat can be stored in the form of adipose tissue, which can be distributed subcutaneously or viscerally, or fat molecules can also be stored intramuscularly or within the liver.[45] Depending on where and how fat is stored, it may serve different but vital functions, including as insulation, as fuel for migration, for overwintering, and for reproduction, and can function as an endocrine organ.[7,9,36,41,45] Fat reserves are thus an important indicator of condition and, ultimately, fitness in vertebrates.[44] Accurate measurement of fat storage is therefore necessary for monitoring animal health.

Among vertebrate groups, nonavian reptiles display a variety of fat storage and use strategies. For example, snakes store the majority of lipids as adipose tissue in paired abdominal fat bodies,[45,53] while lizards often store fat as adipose tissue in the tail.[50] Like most vertebrates, reptiles can also store excess fat in liver tissue, but their liver appears to function primarily as a fat processing site.[45] Seasonal patterns of fat storage and use in reptiles are linked to reproduction and winter dormancy[45] or estivation.[23]

Chelonian (order Testudines) anatomy and physiology present challenges to accurate measurement of fat reserves in live animals. Unlike other reptiles, the majority of a turtle's body is encased within its shell, obscuring visible cues of visceral fat reserves. Furthermore, locations of fat reserves vary extensively among chelonian species depending on habitat and physiological requirements,[9,45] rendering it necessary to investigate species-specific patterns of fat deposition and storage.

From the Department of Natural Resources and Environmental Science, University of Nevada, Reno, 1664 North Virginia Street, MS 186, Reno, NV 89775, USA (Walden, Shoemaker); Veterinary Teaching Hospital, Louisiana State University, Skip Bertman Drive, Baton Rouge, LA 70803, USA (Jania); San Diego Zoo Wildlife Alliance, Veterinary Services, 15500 San Pasqual Valley Road, Escondido, CA 92027, USA (Kinney); Department of Integrative Biology, Oregon State University, 3029 SW Campus Way, Corvallis, OR 97331, USA (Devan-Song); and US Geological Survey, Western Ecological Research Center, 160 North Stephanie Street, Henderson, NV 89074, USA (Drake, Esque). Correspondence should be directed to M. Walden (mar.walden@gmail.com).

Existing methods of measuring fat reserves in turtles include both invasive and noninvasive techniques, all of which have benefits and costs. Lipid extraction from the dried, ground carcass is an accurate but destructive method of measuring total lipids.[24] Cyclopropane dilution for directly measuring lipid components requires an equilibrium chamber and equilibration time for each animal.[19,20] Doubly labeled water can be used *in situ* to calculate total body water by assuming that total body water is a constant proportion of lipid-free mass and thus estimates lipid content by subtraction.[20,39] However, this technique has large errors relative to lipid content.[19] Body condition scores (BCS)[56] based on visual assessment of animals are qualitative and subjective, although they can be done *in situ*. Quantitative body condition indices (BCIs) are computed by regressing mass against body size (e.g. length or volume) and using the residuals as the BCI value for each individual.[37,42,43] However, the utility of BCI is controversial,[17] may be valuable only at the population level, and in tortoises may be obscured by bladder volume.[34] Both BCS and BCI are often treated as a proxy for fat reserves, although this assumption has been tested for only a few chelonian species.[48]

One promising method, computed tomography (CT), has emerged in recent decades as a noninvasive way to accurately and precisely measure fat volume in live animals.[32] This CT technique, which consists of the computational reconstruction of numerous, sequential "slices" of X-ray images with each made across a single plane of the animal, requires only a short time and minimal total radiation exposure. It can produce a high-resolution reconstruction of the 3D whole animal including hard and soft tissue. Determination of total body fat volume of adipose tissue and visceral fat deposits using CT have been validated for multiple taxa, including mammals,[31,32,54] birds,[4] and fish.[51] CT using a soft tissue window has been used to quantify head and neck fat in an embalmed leatherback sea turtle (*Dermochelys coriacea*)[6] and body fat volume in live Eastern box turtles (*Terrapene c. carolina*)[8] and Blanding's turtles (*Emydoidea blandingii*).[40] In addition to measuring fat volume in chelonians, CT is used to diagnose shell and skeletal injuries or disease[1,47,55] and evaluate the coelomic organs,[52] and can also be used to assess hepatic lipidosis.[25,33,47] CT has been described for use in the threatened Mojave desert tortoise (*Gopherus agassizii*) for diagnosis and treatment of illness.[12,27] However, many advantages of CT for the assessment of baseline physiology and anatomy have yet to be explored for this species.

Mojave desert tortoises range across portions of four states in the southwestern United States.[58] This federally threatened species is at risk from multiple environmental and anthropogenic stressors, such as habitat loss and degradation,[58] wildfires,[11,15] subsidized predators,[14] altered diet due to invasive plants,[10] and disease, including upper respiratory tract disease.[58] Standardized health assessments of captive and free-ranging Mojave desert tortoises include a visual assessment of BCS.[59] This protocol was implemented by the US Fish and Wildlife Service (USFWS) to qualitatively assess musculature and visible fat reserves relative to skeletal features of the head and limbs.[28,59] This BCS was found to have a strong positive relationship with relative liver weight[29] but has not been compared with total fat reserves. Quantitative BCI has previously been published for individual- and population-level inferences for Mojave desert tortoises,[13,34] but the relationship between BCI and total fat reserves has not been investigated. The aims of this study were to (1) describe the use of noninvasive CT for measuring body fat volume in Mojave desert tortoises, (2) describe the location of fat reserves, (3) investigate relationships between fat reserves and BCS and BCI, and (4) explore whether relative temporalis muscle depth, measured via CT, correlates with BCS in this species.

MATERIALS AND METHODS

Study animals

Eight captive adult Mojave desert tortoises (six males, two females) were housed individually in outdoor, seminatural 100-m^2 enclosures with two artificial burrows and one or more perennial shrubs and maintained as part of a research cohort for unrelated studies.[2,3] Food (Zoo Med Natural Grassland Tortoise Food, Zoo Med Laboratories Inc, San Luis Obispo, CA 93401, USA) was provided twice weekly and water once weekly during the active season (April–October). These animals exhibited long-term illness from *Mycoplasma agassizii* infection and continued health declines. Research was performed under USFWS Recovery Permit TE-030659 and University of Nevada, Reno IACUC Permit 00710.

Animal evaluation and measurements

Clinical health condition was evaluated, and mass (± 10 g) and shell morphometrics (straight midline carapace length, height perpendicular to the plastron at the suture between the second and third vertebral scutes, and width at the sutures between the fifth and sixth marginals on either side; ± 1 mm) were measured once. BCS was assigned to each tortoise following established protocols.[28,59] Tortoises were first categorized as "underconditioned" (BCS 1–3), "adequately conditioned" (BCS 4–6), or "overconditioned" (BCS 7–9). The assignment of final numerical BCS within the underconditioned and adequately conditioned categories was determined by comparing the apparent height of the temporalis muscle with the apparent height of the sagittal crest, while the presence of subcutaneous fat determined the final BCS within the overconditioned category. This qualitative BCS was assigned to each tortoise independently by two experienced biologists. Observer 1 had more than 15 yr of experience performing visual health assessments of captive and free-ranging Mojave desert tortoises and routinely taught health assessment protocols to professionals seeking to work with the species (KKD). Observer 2 had 5 yr of experience following the cited protocols and was originally trained by Observer 1 (MAW).

Computed tomography

Each Mojave desert tortoise was euthanized as part of a separate research study in November 2018 and immediately transported to the San Diego Zoo (San Diego, CA 92101, USA) for CT. Mojave desert tortoises were positioned in ventral recumbency with the forelimbs and hindlimbs extended and the plastron resting on a 4 cm tall radiolucent foam pad. Internal lasers were used to position the animal isocenter. Whole body CT was performed using helical acquisition at 250 mA and 120 kVp and a pitch of 0.8, on a 32 slice BodyTom CT unit (Neurologica, Danvers, MA 01923, USA). Following image acquisition, the study was reconstructed in a soft tissue (smooth kernel, 400 window width, 40 window level) and bone (sharp kernel, 4000 window width, 500 window level) algorithm at 1.25 mm slice thickness. Windmill correction was performed, and the study was uploaded in DICOM format to a PACS server for evaluation with a DICOM viewer (OsiriX, Pixmeo SARL, Bernex CH-1233, Switzerland).

Temporalis muscle depth measurements were made on transverse CT images using the same DICOM viewer on the bone algorithm, with a bone window (1500 window width, 300 window level). It was not possible during necropsy to measure the depth of the temporalis muscle at the precise location needed and without distorting the height of the muscle due to the anatomical structure of the musculature overlaying the sloping bone surface as well as the skin thickness and toughness. The heads were positioned similarly for every individual for the scans. The temporalis muscle depth was measured in millimeters at its deepest point. The measurement was made perpendicular to the skin in a dorsoventral direction, from the skin surface to the quadrate and squamosal bones (Fig. 1). A multiplanar reformat was performed for each measurement to ensure that the direction of measurement was standardized across individuals. Measurements were taken three times, and the mean and coefficient of variation were recorded.

Distribution of subcutaneous fat and the presence of intracoelomic fat were noted. Measurement of body fat volume (FV) was obtained semiautomatically using segmentation from transverse CT images using the same DICOM viewer. Regions of interest (ROI) were grown by manually selecting an area of fat density using the Grow Region (2D/3D Segmentation) tool. The 3D growing region was applied to the series using Hounsfield unit (HU) threshold ranges of −100 HU and −10 HU to incorporate fat density, with a standard deviation of up to 9 HU to allow for partial volume averaging along the ROI margins. Following application of the threshold, the scan was visually inspected for accuracy, and additional ROIs were made to ensure that all areas of fat density were included. Manual correction to exclude gastrointestinal content with similar attenuation was performed using a brush ROI tool. No liver had negative HU and therefore were not included in the auto-segmentation, which was also ensured by visual inspection. The volume of the fat containing ROIs was calculated in cubic millimeters by the DICOM viewer to determine total body fat. Follicles were not considered as body fat because the attenuation values did not fall within the designated HU range. To calculate the total body volume, an ROI using the Grow Region (2D/3D Segmentation) tool was created of the entire tortoise by excluding the surrounding gas and table surface. The calculated total fat volume was divided by total body volume to calculate body fat percentage.

Necropsy

The locations of visible fat were documented at necropsy. Wet mass of ovaries, follicles, and oviducts was recorded for the two females. If the bladder remained intact after plastron removal (*n*

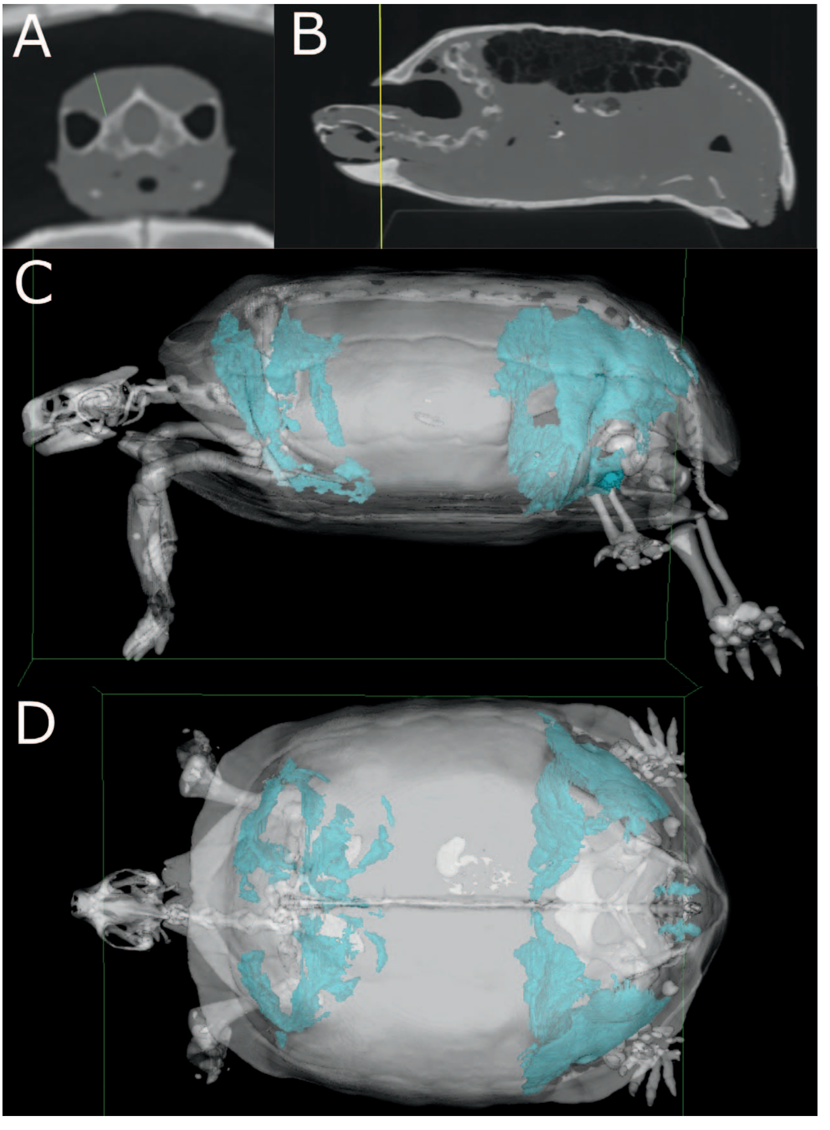

Figure 1. Transverse computed tomography image of the head (A) of a captive adult Mojave desert tortoise (*Gopherus agassizii*) in a bone window (1500 window width, 300 window level) at the level marked with yellow on a sagittal multiplanar reconstruction image (B). The green line on (A) indicates depth measurement of the temporalis muscle, with placement made to measure maximal depth, extending perpendicular to the skin surface in an oblique dorsoventral direction to the skull bones. Sagittal (C) and dorsal (D) views of a computed tomography 3D surface rendering of the skeleton and carapace. Distribution of fat is shown in a blue 3D volume rendering region of interest overlay that was used to calculate volume.

= 7), the bladder was excised, all voidable contents (urine and urates, excluding uroliths) were emptied into a container, and volume was estimated to the nearest 10 ml.

Analyses

Temporalis muscle depth measured by CT was corrected for size by dividing the depth by midline carapace length and multiplying by 100. The relationships between fat volume and BCS and between relative temporalis muscle depth and BCS were quantified with a Spearman's correlation test as a nonparametric analysis appropriate for the ordinal BCS. Slope and intercept of a linear regression of body mass on shell volume (calculated in cubic centimeters, straight midline carapace length × height × width)[38] were calculated from 40

Table 1. Body measurements, assessments, and fat stores calculated with computed tomography (CT) for eight captive Mojave desert tortoises (*Gopherus agassizii*) processed in 2018.[a]

ID	Sex	Obs 1 BCS	Obs 2 BCS	Vol (cm³)[b]	Ellipsoid[c] (cm³)	Mass (g)	TV (cm³)	FV (cm³)	Fat %	Bladder (mL)
1	M	4	4	6,299.9	3,262.0	3,478	3,610.5	35.6	0.99	250
2	M	3	3	4,966.8	2,600.6	2,850	2,845.4	15.81	0.56	350
3	M	4	5	6,324.7	3,311.6	3,556	3,702.8	20.53	0.55	100
4	M	4	4	5,202.7	2,724.1	2,872	2,989.1	14.41	0.48	200
5	M	4	4	4,331.6	2,268.0	3,690	4,142	2.83	0.07	140
6	M	4	4	5,784.42	3,028.7	3,424	3,505.2	23.53	0.67	NR
7	F	4	4	11,550.9	6,048.0	6,280	6,384	145.38	2.28	340
8	F	3	4	4,001.4	2,095.1	2,376	2,392.6	59.74	2.5	160

[a] Obs 1/Obs 2 BCS = observer 1 or observer 2 body condition score; Vol = shell volume; Ellipsoid = body volume; Mass = live mass; TV = total body volume calculated from CT; FV = fat volume calculated from CT; Fat = percentage FV of TV; Bladder = volume of bladder contents.

[b] Midline carapace length × carapace width × shell height.

[c] $(4/3)\pi$[midline carapace length(.5) × carapace width(.5) × shell height(.5)].

captive female Mojave desert tortoises and 40 captive male Mojave desert tortoises processed as candidates for research studies[3] by collaborators in 2013, the same year of intake as the study animals (Esque and Aiello, unpubl. data; Table 3). The BCI of the eight study tortoises was calculated as the difference of observed live mass at shell volume and the expected mass obtained by the linear regression.[38] The same regression was performed on individuals with mass calculated by subtracting the bladder contents for individuals with intact bladders whose volumes were measured during necropsy. Linear regressions were used to test for relationships between fat volume and BCI (including bladder contents) and separately between relative temporalis muscle depth and BCI (including bladder contents) using *F* tests. We set the type I error rate (α) at 0.05 for all statistical tests. Residuals from all six linear regressions described above were tested for normality using Shapiro-Wilk tests. All analyses were performed in R v3.6.2.[46]

RESULTS

Computed tomography

Calculated total fat volumes ranged from 2.83 to 35.6 cm³ (mean 18.8 cm³) for males (0.07–0.99%, mean 0.55% total volume) and from 59.47 to 145.38 cm³ (mean 102.6 cm³) for females (2.28–2.5%, mean 2.39% total volume) (Table 1). The major fat regions identified from CT and consistent among all tortoises were subcutaneous or along the carapace (Fig. 1). Small amounts of intracoelomic fat were reliably observed adjacent to the heart and occasionally observed along the gastrointestinal tract and follicles (Fig. 1). The temporalis muscle was convex and extended beyond the sagittal crest in all tortoises (Fig. 1). Temporalis muscle depth

Table 2. Temporalis muscle depth measured using computed tomography (CT) of eight captive adult Mojave desert tortoises, *Gopherus agassizii*, compared with independent visual assessment by two observers. Numbers in parentheses are coefficients of variation in percent coefficient of variance (% CoV) for the three measurements made using CT repeated for each individual. Midline carapace length was physically measured once per individual prior to CT.

ID	Observer 1 temporalis description	Observer 2 temporalis description	Temporalis muscle depth, mm (% CoV)	Midline carapace length (mm)	Relative temporalis depth (mm/mm)[a]
1	Flush with skull	Flush with skull	9.94 (0.44)	276	3.60
2	Atrophied (concave)	Atrophied (concave)	8.51 (0.31)	249	3.42
3	Flush with skull	Bilobed	10.53 (0.16)	275	3.83
4	Flush with skull	Flush with skull	8.63 (0.42)	253	3.41
5	Flush with skull	Flush with skull	9.81 (0.27)	280	3.50
6	Flush with skull	Flush with skull	10.17 (1.51)	265	3.84
7	Flush with skull	Flush with skull	11.07 (4.62)	329	3.36
8	Atrophied (concave)	Flush with skull	9.41 (0.38)	234	4.02

[a] Temporalis muscle depth divided by midline carapace length, multiplied by 100.

Table 3. Body condition index and trend line calculated using straight midline carapace length and mass for captive adult Mojave desert tortoises, *Gopherus agassizii*.

	Value range	Intercept	Slope
Female (n = 40)	−590.34 to 405.86	−162.46	0.64
Male (n = 40)	−541.81 to 400.11	−54.81	0.58

calculated from CT ranged from 8.51 to 11.07 mm (mean 9.76 mm; Table 2).

Necropsy

The locations of visible fat deposits corresponded to locations of fat identified from CT with the exception that small, isolated intracoelomic fat deposits detected by CT were not recorded during necropsy in a few cases. All tortoises (n = 8) possessed visible fat deposits around the pectoral and pelvic girdles (Fig. 2). Axillary fat deposits were visible in some individuals (n = 4), appearing around the forelimb insertion, immediately anterior to the bridge or posterior to the gular along the edge of the skin (Fig. 2). Inguinal fat deposits were observed in all animals (n = 8), with fat appearing around the hindlimb insertion or along the plastron posterior to the bridge (Fig. 2). Fat was visible at the tail base inside the muscles and at the last thoracic vertebra (n = 3; Fig. 2). Fat deposits were evident in the neck (n = 5), around the heart (n = 1), in the stomach (n = 1), dispersed in the mesentery (n = 4), and around follicles (n = 2; Fig. 2). Small amounts of fat were visible along the carapace or plastron (n = 3; Fig. 2). No discrete, paired abdominal fat bodies associated with the gonads were observed. Discoloration or buoyancy of liver tissue in formalin indicated that fat was likely present in the liver (n = 3; Fig. 2).

Relationship between CT fat volume, temporalis muscle, and BCS

All eight tortoises in this study were assigned a score of 3, 4, or 5 (median 4) out of the 1–9 BCS visual assessment scale. There was no relationship between BCS and fat volume calculated from CT (observer 1: S = 115.75, P = 0.360; observer 2: S = 93.165, P = 0.797; Fig. 3A), and tortoises across the range of body fat percentages were assigned a 4 for the visual assessments (Fig. 3A). Additionally, assignments of BCS varied between observers for two tortoises (Fig. 3A). There was no relationship between BCS and relative temporalis muscle depth from CT (observer 1: S = 105.17, P = 0.547; observer 2: S = 56.51, P = 0.429; Fig. 3B).

Relationship between CT fat volume and BCI

BCI had no strong relationship with fat volume calculated from CT ($F_{1,6}$ = 3.856, P = 0.097, R^2 = 0.39; Fig. 3C). There was also no relationship between BCI and relative temporalis muscle depth measured by CT ($F_{1,6}$ = 0.049, P = 0.83, R^2 < 0.01; Fig. 3D). Bladder contents averaged 6.5% of total body mass (2.8–12.3 ± 3.1% SD). All seven tortoises maintained their condition status ("worse than average" or "better than average") with or without bladder contents relative to the larger captive population. As expected, all tortoise BCI values decreased when bladder contents were removed from mass (Fig. 4).

DISCUSSION

Chelonians provide unique challenges in measuring fat storage and body condition in live animals because of their anatomy and physiology. CT was successfully used to identify fat storage locations and to quantify fat volume in the Mojave desert tortoise. Fat volume calculated from CT did not correlate with the visual assessment of BCS or with quantitative BCI. Furthermore, relative temporalis depth calculated from CT was not related to BCS.

Although CT was performed on euthanized animals in this study, CT should be an effective, noninvasive technique for measuring body fat location and volume in live Mojave desert tortoises. Other studies have shown that no chemical immobilization of live turtles is necessary,[1] and the short time required to complete a scan (30 s) minimizes stress inflicted on the animal. Along with its use in assessing health conditions such as metabolic bone disease or tissue lesions, the ability of CT to assess live Mojave desert tortoise fat volume makes it an invaluable tool for understanding captive and free-ranging populations. However, studies should account for transport and handling times if working with free-ranging endangered tortoises, as well as the availability of equipment and facilities and cost of scans.

Descriptions of fat storage locations in chelonians are rare in the literature, but the function of fat tissue can vary according to location and is thus an important aspect to investigate. Fat deposits in Mojave desert tortoises were observed

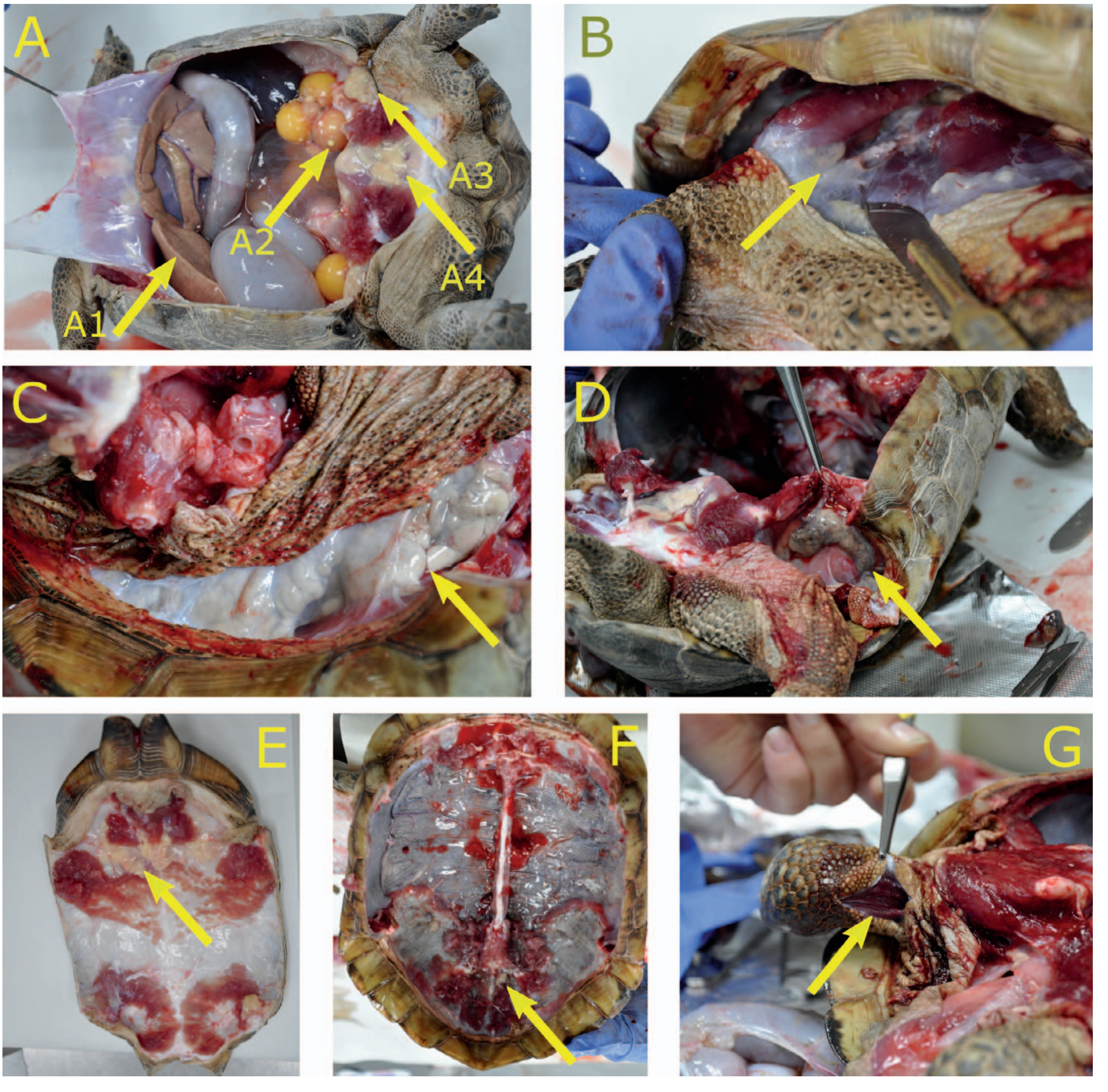

Figure 2. Location of fat observed during captive adult Mojave desert tortoises (*Gopherus agassizii*) necropsy. Arrows point to liver (A1), ovary (A2), inguinal fat (A3) and pelvic girdle fat (A4), tail fat (B), axillary fat (C), inguinal fat (D), plastron fat near left forelimb insertion (E), carapace fat (F), and no visible subcutaneous fat in limbs (G).

at the base of the tail within the tail musculature, which may be unusual for chelonians. Unlike several freshwater turtles, this species did not deposit fat along the bridge of the shell.[57] Consistent with other studies in this species,[29] fat deposits in the axillary area were visible only in individuals with apparently substantial fat in other areas as well, supporting the BCS protocol that assigns the overconditioned category (BCS 7–9) to individuals possessing externally evident subcutaneous fat in that region. Fat associated with organs represented the least common locations and could be a sign of overconditioning attributed to captive feeding and limited enclosure space.[21] Unlike sea turtles, which store fat subcutaneously in their limbs as insulation against cold,[6,18] and snapping turtles,[45] Mojave desert tortoises were not observed to store fat subcutaneously in the limbs. This may render techniques such as ultrasonography to measure fat storage in the limbs (used in sea turtles)[18] futile for this species. Three tortoises displayed fatty liver, not unexpected in captive chelonians, but this condition can also be associated with chronic

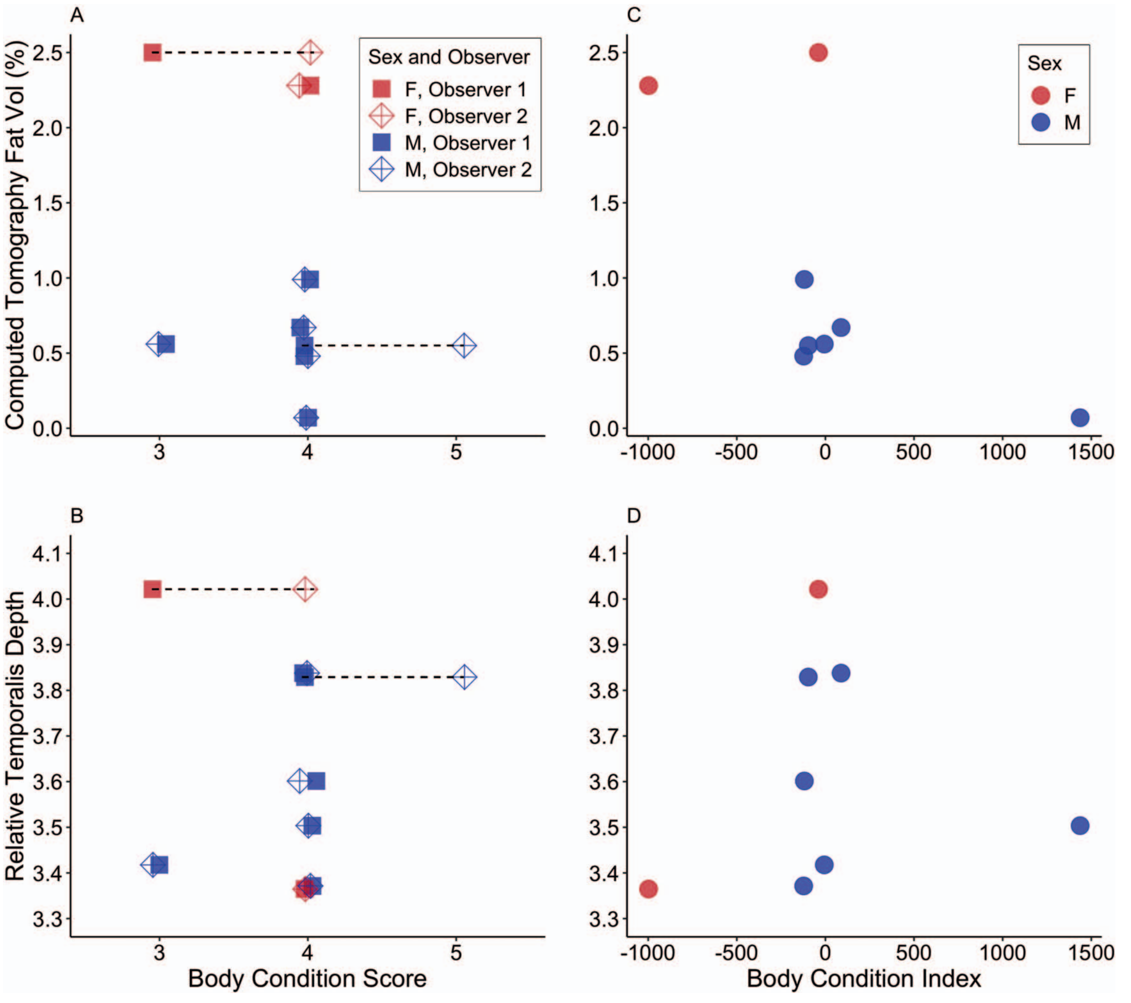

Figure 3. (A) Fat percentage measured using computed tomography plotted against body condition score (BCS; assignments made independently by two observers), (B) relative temporalis muscle depth plotted against BCS, (C) fat percentage plotted against body condition index (BCI), and (D) relative temporalis muscle depth plotted against BCI for eight captive adult Mojave desert tortoises, *Gopherus agassizii*. Points have been jittered for visual clarity. In plots A and B, dotted lines denote differing BCS scores for the same individual (disagreement among the two observers).

disease.[33] Fat accumulation in the liver has been previously documented in this species,[29] and the role of the liver as a major lipid-processing organ has been described in other chelonians.[25,35]

The large difference between male and female fat volume (Table 1) may be related to vitellogenesis, which occurs in the fall for this species,[49] which was when this study was conducted. The seasonal pattern of body lipids previously documented in adult females suggests that females use stored lipids for maintenance during winter dormancy, whereas reproductive effort in spring is fueled by body water and nonlipid dry mass.[20,30] Greater amounts of body fat in adult females relative to males have been demonstrated or suggested to occur in several freshwater and marine turtles,[23,26] but in some species, sex differences in carcass lipids occur only in certain months.[35] For interindividual comparisons, future studies should consider controlling for sex when performing analysis of quantitative body fat metrics for this species.

The body fat volume percentage in this species is similar to body fat volume calculated by CT in Emydidae (Blanding's turtle, 3.41%).[40] Direct measurement of body fat volume or mass from individual body compartments during necropsy was not possible due to the dispersed nature of

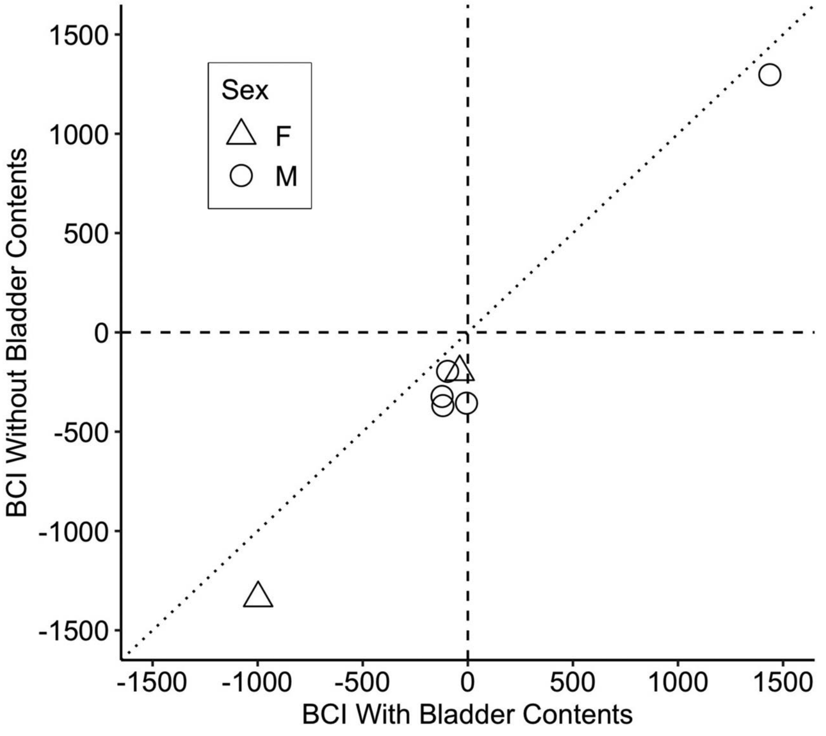

Figure 4. Body condition index (BCI) with bladder contents against BCI with bladder content removed from mass for seven captive adult Mojave desert tortoises, *Gopherus agassizii* ($n = 7$). Vertical and horizontal reference lines represent the lines $x = 0$ and $y = 0$, respectively, and diagonal line represents $y = x$, or a 1:1 relationship for BCI with and without bladder contents.

the visible visceral and subcutaneous fat stores. Also, the need to collect tissue and pathology samples for separate studies eliminated the possibility of comparing CT body fat volume with total lipid extraction of the whole carcass. A previous study that analyzed total carcass lipids in this species reported a range of 1.8–6.7% (g lipids/g body mass) in females.[20] However, caution is necessary when drawing comparisons between CT body fat volume and these previous studies, as total carcass lipids is a mass measurement rather than a volumetric measurement and includes adipose tissue as well as fat molecules stored as intramuscular triglycerides and dispersed in liver tissue.

Visual assessment of BCS in this study failed to correlate with fat volume calculated by CT. This finding may be due to several factors. Each tortoise presented with similar BCS (3–5). A larger sample of tortoises with a wider range of BCS may detect a relationship between BCS and fat volume calculated by CT, as was found previously between BCS and relative liver weights.[29] However, free-ranging Mojave desert tortoises are classified largely with BCS ranging from 3 to 7 ($n = 1{,}055$ individuals, 2,655 encounters, 28 sites, years 2011–2018; Esque, Drake, and Nussear, unpubl. data); therefore, the vast majority of variation in BCS in free-ranging individuals is captured by five integers. BCS may be a better metric for captive individuals, which display a wider range in BCS, or may be representative of health factors not directly related to fat storage in animals with BCS scores 1–6. However, the discrepancies between observers in assigning BCS, including for one tortoise that was assessed as both underconditioned and adequately conditioned, indicates that subjectivity likely plays an influential role in assigning scores in the 1–6 range. The lack of correlation between relative temporalis muscle depth measured from CT and BCS further highlights the complexity of the cranial musculature and skull topology.[60] This complexity likely requires that the BCS protocol be refined to identify more precise landmarks for which point along the sagittal crest and at what distance laterally from the sagittal crest to perform the comparison between temporalis muscle height and sagittal crest height.

This study also determined that BCI calculated using shell volume and body mass may not be a meaningful proxy for fat reserves. Although bladder volume of live animals did not obscure BCI comparisons between study animals and the larger captive population, the greater effect of bladder contents on mass as compared with body fat volume indicates that BCI is likely to be insensitive to fluctuations in body fat reserves. Shell volume was calculated based on a rectangular prism rather than an ellipsoidal model; this choice was made *a priori* because of the small sample size in an effort to maintain consistency with BCI values reported in earlier studies on this species.[38] It is possible that a different method for calculating shell volume and BCI may reveal stronger correlation between BCI and body fat reserves. BCI may yet be shown to have value for interpopulation comparisons but would need further investigation as a proxy for fat reserves in individual tortoises.

The findings of this study have useful impacts on health metrics and understanding body fat storage in Mojave desert tortoises; however, inferences are limited by the sample size (eight individuals). Obtaining free-ranging Mojave desert tortoises to euthanize for validation studies is challenging, but captive individuals may prove useful for obtaining larger sample sizes without further threatening free-ranging populations. Finally, the eight captive animals used for this study were suffering from chronic *M. agassizii* bacterial infection, the causal agent of upper respiratory tract disease,[5] and this sustained illness may have affected the volume or locations of stored fat. However, documented impacts of *M. agassizii* are respiratory and olfactory but do not include appetite.[16,22] This study was performed in November, and calculated fat volumes may not capture the total range of body fat in Mojave desert tortoises during other times of the year.

Despite these limitations, this study has shown the effectiveness of CT as a noninvasive method of describing and quantifying body fat reserves in the Mojave desert tortoise over other established noninvasive metrics to assess body condition, both qualitative and quantitative. Noninvasive methods are necessary especially when studying endangered or rare species in the wild, where vulnerable population status can prevent robust sample sizes using invasive methods. Use of CT is beneficial for assessing captive and free-ranging Mojave desert tortoise health and for studying the ecology and conservation of this threatened species.

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