The evaluation of post-surgical processing on the measurement of cutaneous and myocutaneous biopsy specimen dimension in cats

by

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Abstract

The purpose of this study was to determine if post surgical processing affected the dimensions of skin samples obtained from cats. A second objective was to identify factors that contributed to changes in the dimensions of tissue obtained from normal cats that underwent routine histological processing. Cutaneous and Myocutaneous samples were obtained from twelve normal cats at three locations, the neck, thorax and tibia. Dimensional measurements of the samples were taken at five time points by a single observer. The time points included prior to excision, after excision, after margins were inked, 36 hours after fixation in formaldehyde and after completion of histological processing and hemotoxylin and eosin staining. The measurements at each time point were compared to original measurements at the first time point.

Tissue samples decreased in lateral margins and increased in depth at the final time point. The average shrinkage in the lateral dimensions was 35% and the increase in depth was 55%. The tibia exhibited the greatest shrinkage and the neck exhibited the least shrinkage. Inclusion of the underlying muscle did not affect the degree of change in dimension of the specimen.

In the present study, each element from excision to formalin fixation and histopathological processing induced changes in tissue dimension manifest principally as shrinkage in the lateral margins and an expansion of the depth. Shrinkage should be a consideration when interpreting surgical margins in clinical cases. Further investigation of this phenomenon in a wider feline population in clinical cases is warranted to classify

the degree of change in dimensions of specimens and to identify other variables that affect the degree of tissue shrinkage.

Acknowledgements

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I. INTRODUCTION

Surgical excision is the mainstay of treatment for primary skin neoplasia in cats. Definitive oncologic surgical treatment only requires removal of all neoplastic tissue. However, since the leading edge of the neoplasm is microscopic, the precise edge of an ideal excision cannot be seen. For this reason, the gross neoplasm is removed with additional normal appearing tissue. Since the leading edge of the neoplasm may not uniformly invade the surrounding tissue, the surgical margin is evaluated in the microscopic assessment of the submitted specimen after histological processing to estimate whether it is probable that the entire neoplasm was removed.

The margin status is considered one of the most important components of the veterinary pathology report and is an important prognostic indicator for local disease recurrence and progression.[1-6] In cases where neoplastic cells extend close to the surgical margins, the margins should be quantified based on the smallest distance. The quantification of the surgical margin is directly influenced by the phenomenon of specimen shrinkage.

In humans, specimen shrinkage is a widely acknowledged phenomenon that occurs in tissues that are excised and then undergo histologic processing.[7-18] In an effort to better understand the relationship between in vivo margins and final histopathological margins, there has been investigation of the causal factors into specimen shrinkage. Factors including the tissue type, tissue location, age of the patient, and type of histological processing have been identified to influence the degree of shrinkage.[7,8,12,14,16]

Currently, there is limited information on specimen shrinkage in dogs, and no information in cats.[19] Therefore, interpretation of surgical margins is limited to what can be extrapolated from other species. Due to differences between feline skin and human and canine skin, the extent of tissue shrinkage may be different in this species.[20-22] Specifically there are denser and coarser collagen bundles and larger arector pili muscles.[21] Additionally in the cat, the dorsal neck and scapular regions are constituted by smaller more loosely arrange collagen bundles, which allow greater skin elasticity.[22]

The objective of this study is to investigate the nature of feline tissue shrinkage as it occurs in cutaneous and myocutaneous specimens that undergo routine histological processing. Furthermore, we will identify the time points during histological processing where specimen shrinkage occurs by repeatedly measuring the specimens throughout the entire process from prior to surgical excision to final histopathological measurement and whether other factors such as topographic site or the inclusion of underlying muscle influence the degree of specimen shrinkage.

III. LITERATURE REVIEW

Assessment of Surgical Margins

Examination of surgical margins is based on representative samples (standard size is 5 μm for each section) taken by the pathologist.[23,24] The selection of representative samples is an important factor as a potential source of error for margin evaluation. Optimally, the surgeon would have identified high risk or high interest areas. The pathologist will sample these sites as well as a more general sectioning of the tissue; a process referred to as "cutting in" the tissue, using one of several techniques including cross sectioning (Figure 1), breadloaf technique (Figure 2), breadloaf cross sectioning (Figure 3), peripheral sectioning (Figure 4) and Moh's oblique sectioning (Figure 5).[23] Two of the more common techniques used in veterinary pathology are cross sectioning and breadloaf technique.[24]

Cross Section Technique

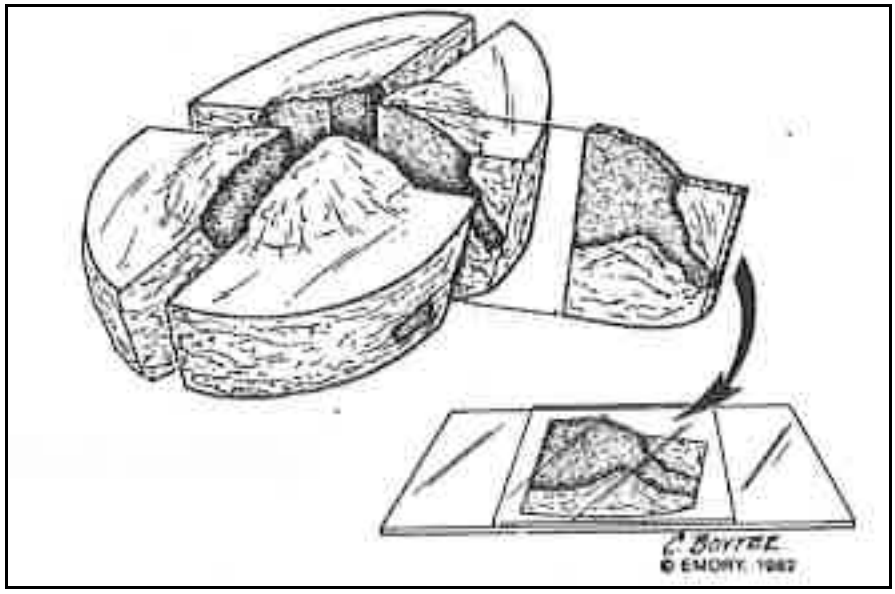

Figure 1. Cross Section, or Cruciate Technique – A section is taken through the short axis of the specimen. A second section is taken at 90 degrees to the short axis, in the long axis of the section. These tissues are further processed for microscopic evaluation.

Breadloaf Technique

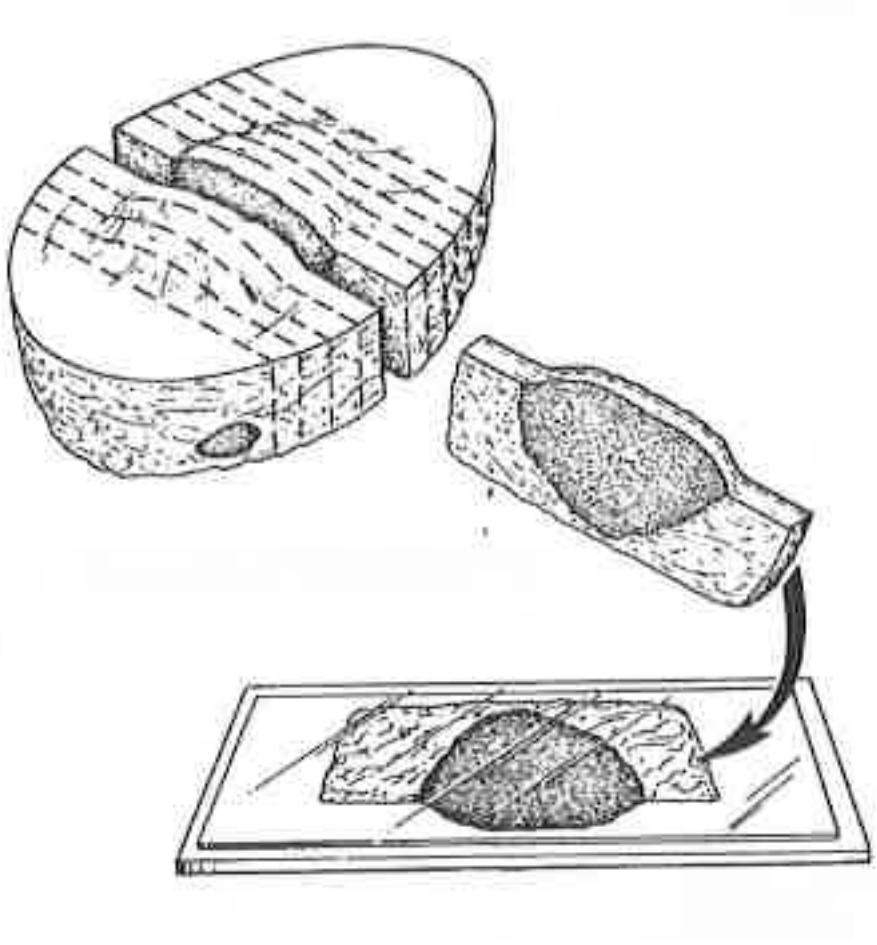

Figure 2. Breadloaf Technique – Serial sections are taken are taken at 90 degrees to the long axis of the specimen. These tissues are further processed for microscopic evaluation.

Breadloaf Cross Section Technique

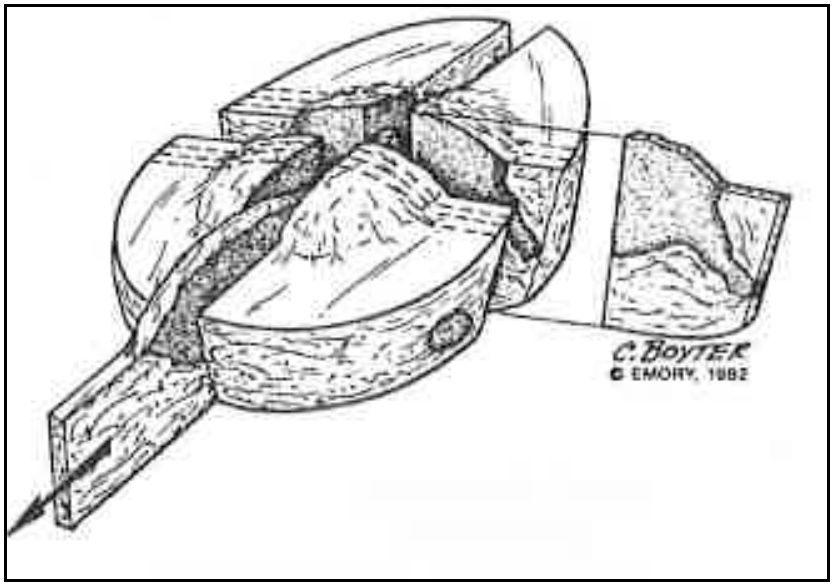

Figure 3. Breadloaf Cross Section Technique – A section is taken in the plane of the long axis of the specimen, and then sequential sections are taken at 90 degrees to the long axis of the specimen in the area of diagnostic interest. These tissues are further processed for microscopic evaluation.

Peripheral Section Technique

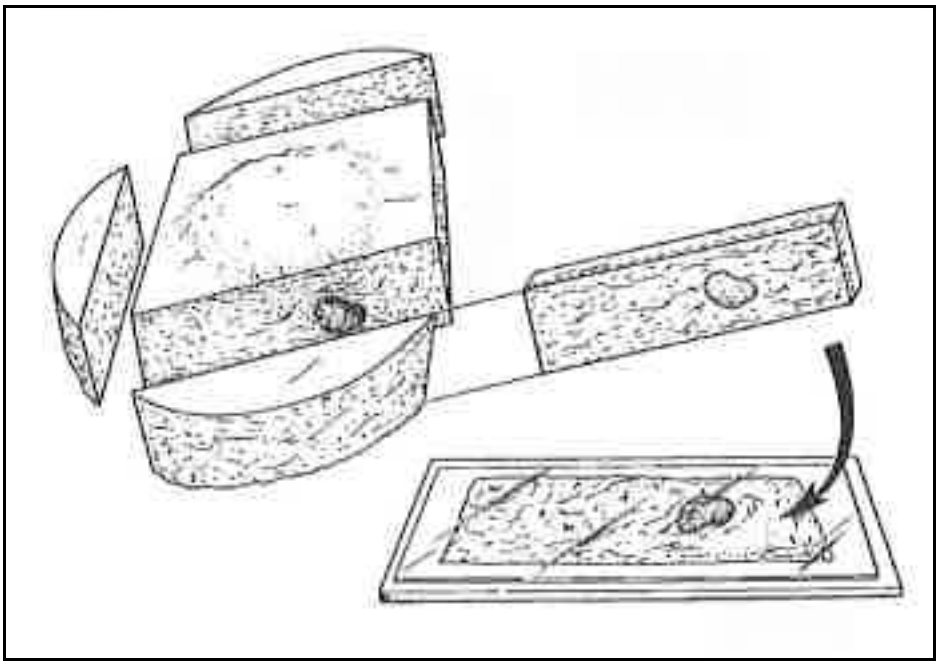

Figure 4. Peripheral, or Perimeter or *En face* Section Technique- Sections are taken from the periphery of the specimen. The cut surface is placed face down on the cassette. This surface will approach the microtome blade. This orientation of the cut surface allows the pathologist request deeper levels of the block to reach the true tumor-free margin. These tissues are further processed for microscopic evaluation.

Moh's Oblique Section Technique

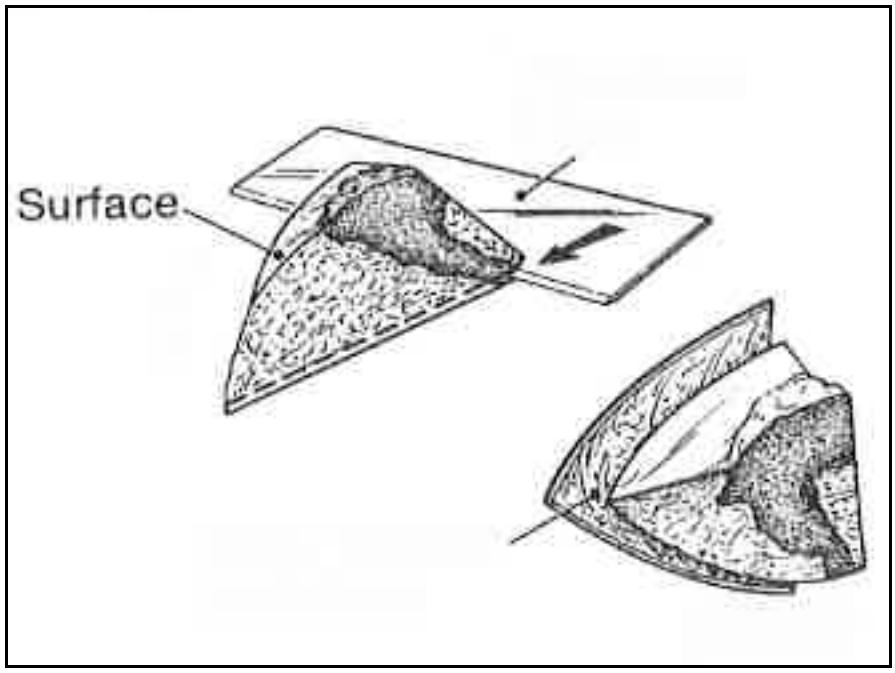

Figure 5. Moh's oblique or *En Face* Oblique Section Technique – The tumor is removed with oblique (45 degree) lateral margins. The specimen is flattened such that the lateral and deep margins are on the same plane and frozen. Microtome sections are then taken in this plane to analyze both the lateral and deep margins simultaneously. If there is evidence of tumor infiltration at this margin, further sections of tissue are taken from the tumor bed.

For Cross sectioning the specimen is sectioned in two planes, 90 degrees to the long axis and then each half is bisected again at 90 degree to the first cut, to create quarters. A slice of tissue is then taken from each of these segments and is further processed prior to evaluation.[24] This technique assumes the tumor is growing symmetrically and is centrally located within the specimen[23] The breadloaf technique involves transversely sectioning the tissue specimen at different intervals[24] The main disadvantage of all techniques is the

relatively small amount of tissue evaluated compared to the volume of the submitted tissue (approximately 1%) and therefore inaccuracies in interpreting the margin status of the excised specimen may be introduced[23,24,25]

To help guide histopathological assessment, the foci of interest can be identified by the surgeon. First, the entire surgical margin may be identified using an adhering marker (latex paint or India's artist ink) to paint the cut surface of the excised tissue to facilitate the assessment of surgical margins by the pathologist.[24,25] Painting the excision margin facilitates distinguishing the true margin and artifactual processing margins created when the tissue is trimmed. Additionally, the surgeon may identify and paint one or more margins of an excised specimen with different colors to permit orientation of the specimen. Finally, the surgeon can identify high risk foci as judged at surgery by placement of suture tags or unique color markers. In combination with a diagram and written description these methods maximally assist the pathologist as the submitted specimen is sectioned for histopathological assessment.

In cases where neoplastic cells extend close to the surgical margins, the margins may be quantified based on the smallest distance from the marker to the neoplasm's leading edge. The pathology report should describe neoplastic cells, the tissue constituents, tissue quality closest to the margin, and an objective measurement of the margin.[24] The definitions of close or narrow margins should be avoided, as there is no consensus on the objective classification of close or narrow margins in veterinary medicine.

Assessment of Shrinkage in Humans

A major source of error in margin determination is tissue shrinkage. Tissue shrinkage is the decrease in dimensions of the tissue following surgical excision and histological processing. Tissue shrinkage is a well- acknowledged phenomenon that occurs following surgical excision and specimen processing.[8-18] The magnitude of tissue shrinkage in a given specimen will directly influence the measurement of the surgical margin reported by the pathologist. Quantification of tissue shrinkage is clinically important in interpreting the histopathological tumor-free margin as this will determine the factor by which this differs from the true in vivo tumor-free margin. For a given neoplasm, the final histopathological margin can be translated back to the true margin in vivo, which can be correlated with the risk of recurrence and outcome.

The focus of previous studies investigating tissue shrinkage has been to identify the main cause of tissue shrinkage and to reliably predict the amount of shrinkage that occurs following excision and processing. The fundamental causes of specimen shrinkage that were identified are twofold. Firstly, the retractile properties of tissue lead to shrinkage prior to fixation.[8,12,14] Secondly, specimen processing further causes shrinkage of specimens.[7,8,12,16] There was a debate as to whether the majority of shrinkage occurs after surgical excision and prior to formaldehyde fixation or after histopathological processing.

Recent papers identify the majority of shrinkage to occur prior to fixation[812,14] In excised skin specimens, 70-100% of total specimen shrinkage occurred after skin excision and prior to fixation.[7,12] The shrinkage post-excision is attributed to intrinsic contractile

properties of the tissue itself.[14] The retractile properties of human skin specimens have been further investigated.[15]

In one study, in vivo thickness of the specimen was measured using ultrasound and compared to the ex vivo measurement. A significant increase in thickness was found to correspond to the decrease in the width and length of specimen, suggesting that the specimen retracted following excision[15] The amount of contraction and therefore shrinkage is dependent on the component tissues included in the specimen.

Intuitively it would seem that the mechanical properties of the tissue relating specifically to the collagen and elastin content would influence the contraction and shrinkage of the specimen. However, this has yet to be unequivocally proven.[7]

Patient factors such as the patient's age can indirectly affect tissue shrinkage due to the influence on inherent tissue contractility. However, there is little consensus amongst human studies as to whether age significantly affects specimen shrinkage, as most studies using cut off points of 50 or 60 years, not able to find an association.[7,8,14] Furthermore, the clinical significance of a small association between increasing age and decreasing specimen shrinkage is unknown.

In addition to the inherent qualities of skin, other factors can play a role in specimen shrinkage, including tumor-related factors such as specimen size and location. Specimen size can affect the magnitude of shrinkage with larger specimens having a relatively

lower amount of shrinkage.[8,12] An inverse relationship exists between the initial length and depth and the degree of shrinkage of the specimen[8] Topographic location of the lesion is another important determinant of the extent of tissue shrinkage. Tissue located on the limbs is more likely to shrink when compared with those of the head and neck.[14] This is most likely associated with the relative elasticity and inherent contractility of the tissue after excision.

Early studies assumed tissue shrinkage was uniform across the entire specimen. Day and Lew estimated that the original margin could be calculated by applying a 25% shrinkage factor to cutaneous specimens that are excised and processed.[26] Goldstein reported doubling the final processed margin length to estimate the in vivo margin.[11] However, recent data suggests that non-neoplastic tissue may shrink more than neoplastic tissue. Blasdale identified a differential shrinkage between normal tissue, which had mean shrinkages of 19% and by 11%, respectively.[7] Similarly, Hudson and Peacock detected an 8% difference in the amount of shrinkage between benign and malignant tumors.[13] This is hypothesized to occur due to the inflexible structure of tissue protein, lipid and water in neoplastic tissue, which allows the tissue to retain the original shape.[7] Therefore calculations that are based on a derived formula or shrinkage factor may underestimate the magnitude of the in vivo tumor free margin.

The problem of non-uniform reduction in tissue shrinkage at the transition zone of neoplasm-normal tissue was further highlighted when margins of breast cancer surgery were assessed.[18] The tumor shrinkage (4%) was significantly lower than adjacent non-

neoplastic tissue (34%). The explanation the authors offered was suggested the possibility of degradation of lipids by formaldehyde during the fixation process, leading to greater shrinkage of the non-neoplastic tissue, which has a higher fat content. Once again this highlights how reported margins may be spuriously lower than the in vivo margin.

Current Veterinary Literature

There is limited information on tissue shrinkage following excision from canine or feline specimens. Although pathologists accept that this phenomenon does occur in canine and feline patients, the majority of this information has been extrapolated from the human literature.[24]

One study investigated the effect of routine processing on tissue specimens from dogs. In this study a significant decrease in specimen width and length was evident and similar to the human studies, there was an increase in specimen depth. The cause of this change was attributed to tissue processing. Samples that consisted of skin and subcutaneous tissue had a greater degree of shrinkage compared to samples that also contained muscle.[19]

Few studies have investigated the histological assessment of surgical margins in dogs. These studies have investigated the relationship between histopathological margins and recurrence of cutaneous mast cell tumors.[2,4,6] In these studies close margins were defined as those ≤1mm. This was an arbitrary cut off point, and there is no data to indicate a histological margin of ≥1mm is a complete margin. In contrast, a similarly designed study on a cohort of dogs with mast cell disease found lateral margins that exceeded

≥10mm and deep margins that exceeded >4 mm had no evidence of recurrence.[5] These numbers resulted from measuring processed tissues so the quantity of tissue that is needed to be harvested as the original margin is not correlated and the exact preoperative measurement required for clean or close margin remains unclear.

Additionally, definitions of margin status will vary with tumor type and behavior. In a study on cutaneous tumors in dogs and cats, which consisted of three cutaneous tumor types including soft tissue sarcoma, mast cell tumors and carcinomas, there was a difference in the accuracy of a < 2mm margin to predict the likelihood of recurrence in each tumor type.[27] The data from this study indicated that there was a 76-94% correlation between margin classification (dirty or close vs clean) and recurrence of tumor. However, the variability of tissue shrinkage and its influence on the final measurement of the tumor free margin, and therefore the risk of recurrence remains unknown in cats.

Conclusions

Current understanding of tissue specimen shrinkage recognizes that the majority of shrinkage occurs following excision. Specimen shrinkage is attributed to the retractile properties of the tissue that result in shrinkage of the length and width of the specimen, and may result in a marginal increase in the depth of the specimen. Although formulas have been developed to predict the in vivo measurements, discrepancies between the relative shrinkage of tumor vs non-tumor tissue within a specimen may be introduced by pre-processing marking, processing artifact, topography and relative content of neoplasm. Such factors may falsely decrease or increase the size of the surgical margin calculated.

There is currently little data in the veterinary literature that allows the pathologist to accurately predict the amount of shrinkage that occurs from surgical excision to final histopathological assessment of surgical margins. This presents a dilemma for the clinician in interpreting surgical margins and advocating further treatment recommendations.

III. MATERIALS AND METHODS

Animal Subjects

Twelve adult cats with a body condition score of 4 to 6 (on a scale of 1 to 9) were included in the study. Cats were obtained upon euthanasia after completion of another unrelated study. The skin and underlying tissues were not disturbed in the regions of sample collection. Cats weighed between 2.4- 4.8 kg (mean= 3.27 kg). On physical examination prior to euthanasia, cats were free of any grossly apparent dermatological disease.

Skin Sample Collection

Cats were placed in lateral recumbency and six areas were routinely clipped. Skin samples were obtained from three sites bilaterally (6 samples/cat Figure 6). The sites determined for sampling were the lateral aspect of the neck, lateral aspect of the thorax, and the proximolateral aspect of the tibia. The samples collected from the neck were centered over a point equidistant between the point of the scapula and the wing of the atlas. The samples collected from the lateral thorax were centered over a point 5 cm caudal, and at the level of the point of the elbow. The samples collected from the tibia were 2.5cm caudal at the level of the tibial tuberosity.

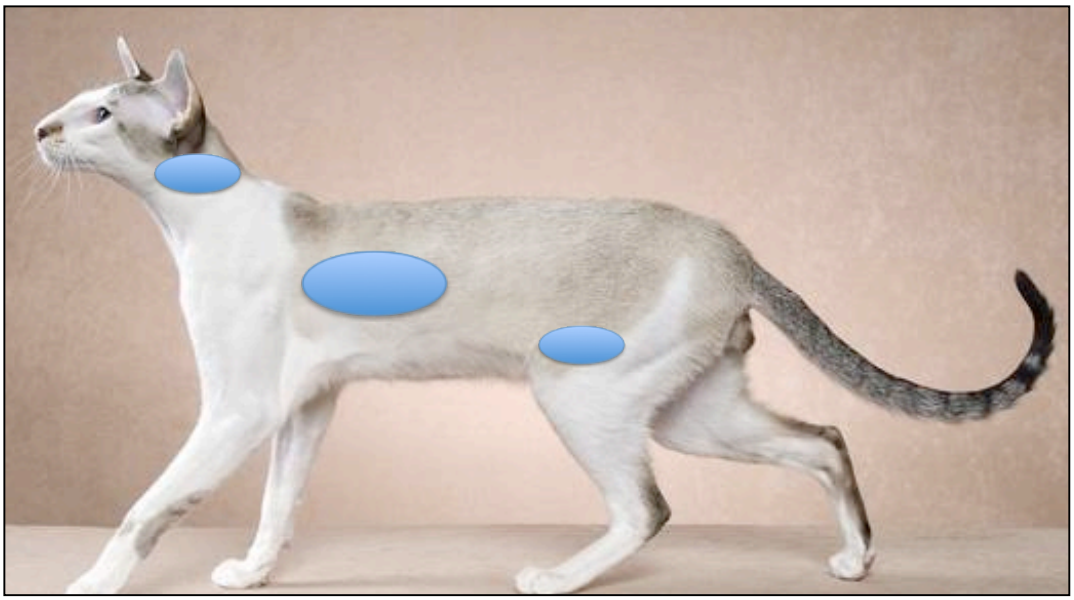

Figure 6. Illustration of sites (lateral neck, lateral thorax, proximolateral tibia) of specimen collection. Specimens were collected bilaterally in 12 cats (6 samples/cat).

All samples were elliptical and orientated in the diagram (Figure 6). The samples collected from the neck and tibia measured 80mm x 40mm in the craniocaudal and dorsoventral plane, respectively. The samples collected from the lateral thorax measured 120mm x 60mm in the craniocaudal and dorsoventral plane, respectively. The deep plane was taken to include dermis and subcutaneous tissue and fascia. In the samples collected from the lateral thorax, the left or right side was randomly assigned, by coin toss, to include the underlying latissimus dorsi.

A plastic template was used to draw the ellipse over each location using a surgical skin marker (Devon Surgical Skin Marker, Covidien, Mansfield MA). The skin was incised with a No. 10 scalpel blade. The initial skin incision was made to the desired depth in one section to allow the depth to be measured prior to contraction of the specimen. The desired depth was to the level of the fascia in the neck and tibia locations. In the thorax

location, the desired depth was at the level of the fascia or included latissimus dorsi. The tissue was then undermined and the excision was completed by incising the skin, subcutis and muscle (where indicated) with Metzenbaum scissors.

Measurements

The tissues were measured and recorded in triplicate to the nearest 1mm using a ruler (Devon Skin Marker Ruler, Covidien, Mansfield MA) by a single observer and the mean was calculated. Once the specimen was excised, one drop of tissue ink (Margin Marker, Vector Surgical, Waukesha, WI) was used to mark the cranial, caudal, dorsal and ventral aspects of each skin sample. This allowed future orientation and identification of each margin and served as a repeatable point of measurement. The color selected for each region was standardized for consistent orientation during subsequent measurements. Specifically, the tissue ink was used to mark the cranial margin yellow, the caudal margin red, the dorsal margin blue and the ventral margin green.

The measurements included cranial to caudal distance (length) and the dorsal to ventral distance (width) and the distance from the surface of the skin to the level of the deepest tissue layer excised (depth).

The measurements were sequenced in the following manner: Once the proposed incision was marked measurements of the length and width were taken (time point, T1). After the skin incision was made to the desired depth, the depth measurement was taken (time point, T1). After completion of excision, samples were placed on a flat glass surface with

1-2mL of sterile saline added to reduce surface tension and then the dimensions were measured (time point, T2). The sample was then inked, and 10 minutes was allotted for drying, prior to completing the third measurements (time point, T3). Samples were then placed in 10% neutral buffered formaldehyde that was approximately 10 times the sample volume for 36 hours. Following formaldehyde fixation, measurements were then taken (time point, T4). Final measurements were taken after completion of routine histological processing with standard paraffin embedding and hemotoxylin and eosin staining (time point T5). Each slide was scanned, and measurements were taken using digital pathology software (Visiomorph DP, Visiopharm, Denmark) by the same observer (Figure 7).

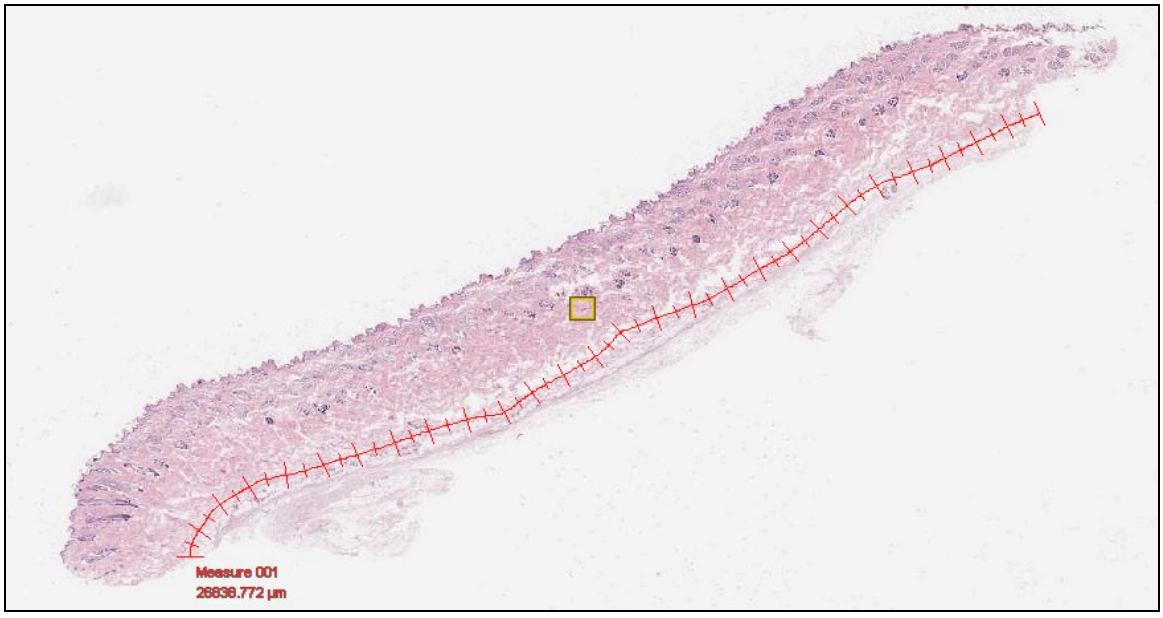

Figure 7. Measurement of skin sample at T5. The slide was scanned and the dimensions were measured digitally using software (Visiomorph DP).

Data recorded included 3 measurements (length, width and depth) at 6 anatomical sites (lateral neck, lateral thorax and proximolateral tibia bilaterally) at each of the 5 time

points. Each data set was compared to the original measurements based on percentage change recorded as a positive or negative.

Statistical Analysis

The data was analyzed using Statistical Analysis System (SAS, Version 9.3, Cary, NC), and the mixed model for repeated measures analysis to evaluate the effects of anatomical site, time point and dimension was employed. The effect of time point on skin specimen dimensions was assessed using least means square test for multiple comparisons. The effect of location was evaluated between samples from the neck, lateral thorax (without the inclusion of the underlying muscle) and proximal tibia after normalizing data to the original size using the Scheffe's test for multiple comparisons. The effect of including a muscle layer was determined via comparisons of samples from the lateral thorax using the Scheffe's test for multiple comparisons. Values of $P < 0.05$ were considered significant.

IV. RESULTS

Animal Subjects

Skin specimens were obtained from twelve cats at three locations bilaterally (total of 72 samples). All cats were 14 months of age and were female. Cats had a mean weight of 3.27kg, and a mean body condition score of 4/9. There were no dermatological conditions noted on physical examination.

Measurements

Effect of Time

There was a significant decrease in the length and width of skin specimens from T1 to T5 ($P<0.001$). There was also a significant increase in the depth from T1 to T5 at the lateral thorax, tibia and neck locations ($P=0.0116$, $P<0.001$, respectively)

Change in original Length (%) of specimens vs time

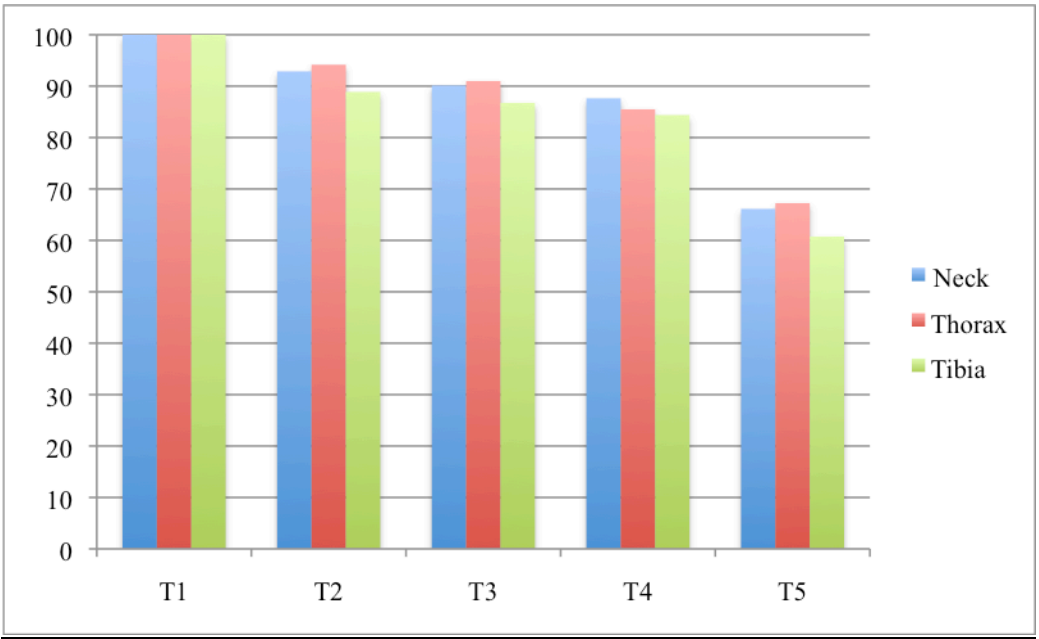

Figure 8. Graph of the change in length (%) of the skin specimens over time. The x-axis represents the time points, and the y-axis represents the change in length as a percentage of the original dimension in vivo.

Change in Length of specimens from original dimension (%)

Location	T2	T3	T4	T5
Lateral Thorax	-5.9	-9.0	-14.5	-32.7
Neck	-7.2	-12.4	-12.4	-33.8
Tibia	-11.1	-15.6	-15.6	-39.3

Change from original Width (%) of specimens vs. time

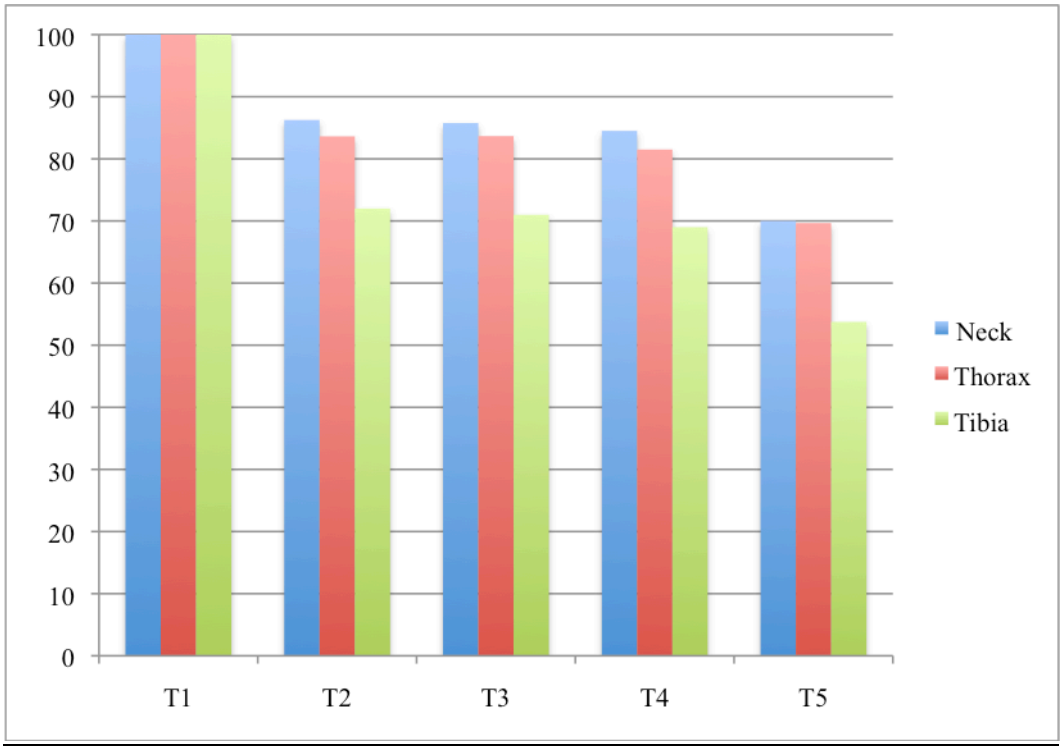

Figure 9. Graph of the change in width (%) of the skin specimens over time. The x-axis represents the time points, and the y-axis represents the change in width as a percentage of the original dimension in vivo.

Change in Width of specimens from original dimension (%)

Location	T2	T3	T4	T5
Lateral Thorax	-15.1	-16.4	-18.4	-30.3
Neck	-13.7	-14.2	-15.5	-30,0
Tibia	-28.0	-29.0	-30.9	-46.2

Change in original Depth (%) of specimens vs time

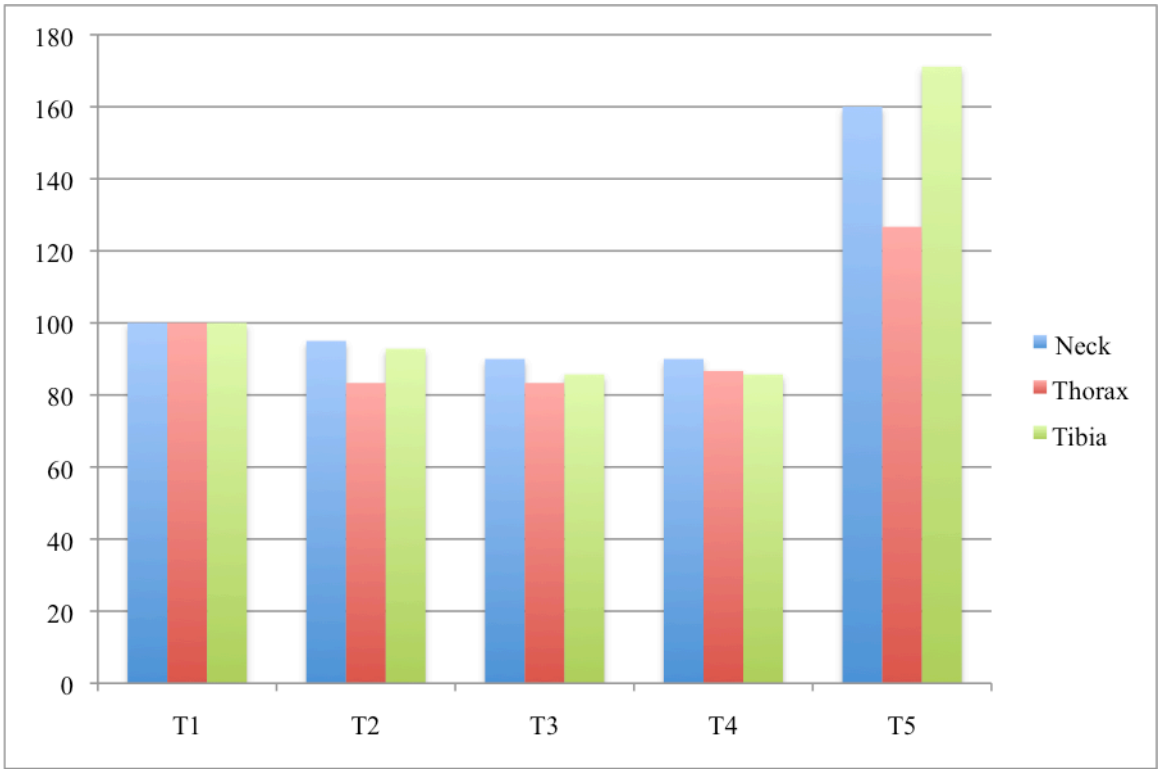

Figure 10. Graph of the change in depth (%) of the skin specimens over time. The x-axis represents the time points, and the y-axis represents the change in depth as a percentage of the original dimension in vivo.

Change in Depth of specimens from original dimensions (%)

Location	**T2**	**T3**	**T4**	**T5**
Lateral Thorax	-15.9	-15	-13.1	+27
Neck	-6.9	-8.3	-8.3	+62.3
Tibia	-8.1	-14.1	-22.2	+75.8

Effect of plane

The magnitude of decrease in the width of specimens was greater than the magnitude of decrease in length, at time point T2 through T4 ($P<0.001$). However, at time point T5, there was no difference in the magnitude decrease in width compared to length of the skin specimens ($P=0.5849$). The change in size of skin specimens in the depth plane was different from the width plane at time points T2 through T5 ($P<0.001$). The amount of change in size of specimens in the depth plane was only different from the length plane at T5 ($P<0.001$).

Effect of Location

The neck, lateral thorax and tibia locations exhibited different magnitude of change in width and length at time points T3, T4 and T5 ($P= 0.413$, $P<0.001$, $P<0.001$) but not at T2 ($P= 0.0789$).

On pair wise comparison of the magnitude of decrease in length and width, the specimens of the neck and tibia were significantly different at T3, T4 and T5 (P 0.0422, $P<0.001$, $P= 0.013$). However, there was no significant difference between the magnitude of shrinkage on the neck compared to the lateral thorax.

Effect of Inclusion of Underlying Muscle

Change of Skin Specimen from original size (%)

Location	Plane	T1-T2	T2-T3	T3-T4	T4-T5
Lateral Thorax	Length	-5.9	-9.0	-14.5	-32.7
Lateral Thorax – inclusion of muscle		-13.4	-16.7	-16.7	-35.5
Lateral Thorax	Width	-15.1	-16.4	-18.4	-30.3
Lateral Thorax – inclusion of muscle		-17.9	-18.7	-20.2	-32.2
Lateral Thorax	Depth	-15.9	-15	-13.1	+27
Lateral Thorax – inclusion of muscle		-10.6	-8.0	6.2	+22.2

There was no difference in the depth or width of skin samples at time points T1-T5 skin samples that included the underlying muscle compared to control on the lateral thorax (see table below). There was a significant difference in the length measurement at T2 between skin samples that included the underlying muscle compared to the control. At

time points T1, T3-T5, there was no difference in the length measurement between skin samples that included the underlying muscle compared to control.

Geometric Least Means Procedure for comparison between Lateral Thorax samples and Lateral thorax with underlying muscle samples

Plane	**Time Point**	**Difference between Means**	**Simultaneous 95% Confidence Interval**
Depth	T1	-0.1667	(-0.8127, 0.4693)
	T2	-0.3056	(-0.8844, 0.2733)
	T3	-0.3611	(-0.9200, 0.1977)
	T4	-0.3611	(-0.8479, 0.1257)
	T5	-0.0607	(-0.9849, 0.8636)
Length	T2	-9.056	(-17.916, -0.195)*
	T3	-9.250	(-18.706, 0.206)
	T4	-6.611	(-16.250, 3.028)
	T5	-3.376	(-12.371, 5.618)
Width	T2	-1.667	(-4.5471, 1.2138)
	T3	1.3889	(-4.4054, 1.6276)
	T4	1.0556	(-41844, 2.0733)
	T5	1.143	(-7.083, 4.797)

* Denotes statistical significance

V. DISCUSSION

The present study confirms there were substantial alterations in dimensions of skin samples following excision and histological processing in cats. This study was closely modeled on the canine study to allow for comparisons. Similar to the canine study, there was a decrease in size of skin samples in the length and width dimensions and an increase in the depth dimension.[19] The underlying cause of this effect could be associated with the excision and manipulation of tissues and the inherent retractile properties of skin.[8,12,14] Additionally, dehydration of the specimen with immersion in alcohol and the fixation process may lead to further changes to the structure of the skin sample.[7,12,16]

The length and width of the skin specimen, otherwise known as the lateral margins, decreased on average by 35.3% and 35.5%, respectively, following excision and histological processing. However, the depth of the skin specimen on average increased by 55%. This is similar to the trend reported in dogs, where the length and width decreased by 26.5 % and the depth increased by 65.3% (Reimer, *Am J Vet Res* 2005). The decrease in length and width of skin specimens is consistent with similar studies in humans, where normal lateral margins decreased in size between 15- 25% of the original dimension.[7,12,16] However, the increase in the depth of tissue measured has not been consistently found in humans.

The depth measurement in the present study was made similar to the other measurements. It was difficult to accurately measure the depth in situ prior to completion of the excision.

The measurement at T1 was used then to calculate the decrease from original dimensions, and therefore could have been a source of measurement error, and the calculation of overall change in each time point thereafter. However, it is still plausible that the specimens retracted in the lateral dimensions and increased in depth after histological processing. This finding was also noted in the canine study, where the specimens were thicker in the depth plane whilst smaller in the width and length planes[19] This may be due to removal of water, lipids and alterations in the structure of cell proteins by the fixation and dehydration process that may have caused the epidermis, dermis, subcutaneous structures and underlying muscle to separate.[28]

In clinical cases, the measurement of tumor depth and the surgical margin in this plane is interpreted alongside the presence of a fascial plane. The presence of fascia may act as a biological barrier in some cases and is therefore is evaluated in the determining the completeness of excision.[29] In the current study, the margin in the depth plane increased following excision and processing compared to the in situ margin. The over estimation of this margin is a consideration when interpreting the reported depth margin in the histopathology report. However, this should be combined with the assessment of the presence of one or more fascial planes present in the surgically excised tissue.

The neck, lateral thorax and tibia were chosen as locations, to allow comparison to the canine study as well to represent three distinct locations. The tibia had a significantly greater amount of shrinkage compared to the neck, in the lateral dimensions at time points T3-T5. The lateral thorax was intermediate in the amount of shrinkage, similar to

the canine study[19] This finding in the present study is consistent with other human studies that have shown that the extremities exhibit a greater amount of shrinkage compared to the trunk.[13] In humans this is thought to be due to the inherent contractility of tissue after excision.[14] However, this theory is less plausible in the present study where the T4 and T5 time points exhibited the greatest difference, rather than the T2 time point, suggesting that the tissues from the tibia underwent a greater degree of shrinkage during the cross-sectioning, fixation, dehydration, microtomy, embedding and staining.

The long axis of the ellipse was oriented in the craniocaudal plane. This was opposite to the lines of tension in the neck and lateral thorax location.[30] Although this does not typically mimic the clinical situation, this orientation was chosen to compare results to the previously reported canine study. In the canine study, the majority of the total shrinkage occurred in the plane of tension, since the short axis was orientated in the plane of tension.[19] However, in the present study, the total shrinkage in both planes was similar, suggesting that the plane of tension did not influence the magnitude of shrinkage.

The majority of changes occurred between T4 and T5. Specimens were cross-sectioned, placed in alcohol solution, embedded in paraffin and microtome sectioned, mounted on the slide and stained with hemotoxylin and eosin. These steps are responsible for the majority of changes to the specimens in the present study. The dehydration process where tissues are immersed in alcohol can lead to rapid removal of water from the specimen, which correlates with the degree of shrinkage[28] In the present study, the specimens were placed in gradually increasing concentration of alcohol fixative for a fixed time period

that had been previously calibrated for the automated processor. Additionally, the embedding of specimens in paraffin is performed at a higher temperature, which affects the structure of collagen and leads to distortion and shrinkage of the specimen.[31]

The inclusion of the underlying muscle at the lateral thorax location did not influence the alterations in dimensions. This finding is surprising, given the canine study showed that inclusion of the underlying muscle reduces specimen shrinkage. One explanation could be a type 2 error in this study, however, given the larger sample size in this study this is unlikely. In the canine study, Labradors were chosen, with a larger amount of subcutaneous tissue and a more robust cutaneous trunci and latissimus dorsi muscle; it is likely that the depth of this sample compared to the control group was greater. In the present study, cats were of moderate body condition, however the cutaneous trunci and latissimus dorsi are relatively thinner, and therefore the influence of these muscle on the magnitude of shrinkage may have been smaller.

In the present study a scalpel blade and scissors were used to excise the tissue. Other modalities such as cutting diathermy, coagulation diathermy, carbon dioxide laser, or harmonic scalpel may be used to excise the cutaneous neoplasm. These techniques have been known to induce cellular damage, including condensation, hyalinization and loss of fibrillar texture of collagen, at the surgical margin.[32] Additionally, the thermally induced contraction of collagen resulted in irregular shrinkage patterns.[32] In a previous study investigating different cutting modalities, cutting diathermy produced the cleanest cut with the least amount of shrinkage.[33] Comparatively the scalpel produced the greatest

amount of shrinkage.[33] Therefore, this data may not be extrapolated in clinical cases where other methods were employed during the surgical excision.

Study Limitations

The present study has some limitations. Firstly, the study involved a small number of cats that are not representative of the wider feline population. The age, breed and size were homogenous. Extrapolating this data to other cats may not be accurate. Different breeds, particularly with different skin elasticity (for example, the Devon Rex) may experience a variation in skin shrinkage during histological processing. Additionally, patients that are more likely to have neoplastic conditions may be older, and may experience a different degree of skin shrinkage. In humans, patients older than 60 years of age had decreased shrinkage compared to patients that were younger.[16]

The study was performed in recently euthanized cats. All skin samples were collected within 30 minutes of euthanasia. Although unlikely to influence the degree of shrinkage, there may have cell autolysis and decomposition, which may influence the degree of skin shrinkage. Following death, the dermis and epidermis do not undergo any histological alterations in the first 6-8 hours.[34] Furthermore, in the present study there was no evidence of cell autolysis on evaluation of the specimens at T5.

Another source of error is the standardization of tissue sample collection and measurement. Distortion of the skin during was minimized by using a template to draw the proposed skin incision. Additionally, skin samples were manipulated minimally

during the collection and measurement. To eliminate interobserver error the samples were measured by a single observer. The observer was not blinded to the treatment, and this remains a potential source of bias.

Although the study was conducted in cats with normal skin that were free of dermatological conditions, the data can be extrapolated to patient with neoplastic conditions of the skin, considering that the tissue of interest when assessing and measuring the surgical margin should be non-neoplastic tissue. However, it is possible to have inflammation surrounding neoplastic tissue, which may exhibit a variation in the pattern of shrinkage.

VI. CONCLUSION

The purpose of this study was to evaluate any changes to the size of skin specimens that underwent excision and routine histological processing in cats and to compare these changes to those reported in the dog. To the extent practical, the methods used were to duplicate the dog study. The findings of these two studies were similar with differential magnitudes. The lateral dimensions decreased the depth of specimens increased from each site: the neck, lateral thorax and proximal tibia. A greater amount of change from the original dimensions was noted in the specimens from the proximal tibia. Although changes occurred following excision, most of the changes to the dimensions of samples occurred following processing including the steps of dehydration, microtome sectioning, paraffin embedding and rehydration. The magnitude of decrease in dimensions in the lateral margins is in the order of 35% and the magnitude of increase in depth dimensions is in the order of 55%. Although this data may help guide interpretation of surgical margins in cats, further investigation of this phenomenon in clinical cases in a wider population of cats is required.

VII. REFERENCES

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VIII. APPENDICES

APPENDIX 1

Data

Obs	Cat	Location	Plane	Side	T1	T2	T3	T4	T5
1	14	Neck-no muscle	Width	Left	40.000	35.000	35.333	35.667	25.649
2	14	Neck-no muscle	Length	Left	80.000	71.000	70.333	69.000	53.447
3	14	Neck-no muscle	Depth	Left	2.000	2.000	2.000	2.000	4.502
4	14	Neck-no muscle	Width	Right	40.000	33.667	34.000	34.000	27.320
5	14	Neck-no muscle	Length	Right	80.000	72.000	70.667	69.667	49.230
6	14	Neck-no muscle	Depth	Right	2.333	2.000	2.000	2.000	2.980
7	14	Lat Thorax-no muscle	Width	Right	60.000	49.333	48.667	48.333	43.893
8	14	Lat Thorax-no muscle	Length	Right	120.000	93.667	92.333	91.333	70.200
9	14	Lat Thorax-no muscle	Depth	Right	3.667	3.000	2.667	2.667	4.410
10	14	Lat Thorax-muscle	Width	Left	60.000	44.333	45.000	45.667	39.547
11	14	Lat Thorax-muscle	Length	Left	120.000	94.333	93.333	93.333	73.318
12	14	Lat Thorax-muscle	Depth	Left	3.667	3.667	3.667	3.667	5.420
13	14	Tibia-no muscle	Width	Left	40.000	27.667	27.667	27.333	19.761
14	14	Tibia-no muscle	Length	Left	80.000	66.000	65.667	65.000	45.260
15	14	Tibia-no muscle	Depth	Left	1.333	1.333	1.000	1.000	3.599
16	14	Tibia-no muscle	Width	Right	40.000	30.667	31.333	30.667	23.970
17	14	Tibia-no muscle	Length	Right	80.000	74.333	73.667	74.000	53.645
18	14	Tibia-no muscle	Depth	Right	1.333	1.000	1.000	1.000	2.658
19	13	Neck-no muscle	Width	Left	40.000	35.333	35.000	35.000	28.450
20	13	Neck-no muscle	Length	Left	80.000	75.667	74.333	74.000	49.744
21	13	Neck-no muscle	Depth	Left	1.333	1.333	1.333	1.667	2.570
22	13	Neck-no muscle	Width	Right	40.000	32.333	33.333	33.000	28.990
23	13	Neck-no muscle	Length	Right	80.000	78.667	77.333	76.667	58.738
24	13	Neck-no muscle	Depth	Right	1.667	1.333	1.333	1.333	2.720
25	13	Lat Thorax-no muscle	Width	Left	60.000	48.000	48.667	48.333	17.177
26	13	Lat Thorax-no muscle	Length	Left	120.000	118.333	117.000	116.000	76.840
27	13	Lat Thorax-no muscle	Depth	Left	3.000	2.000	2.000	2.000	3.042
28	13	Lat Thorax-muscle	Width	Right	60.000	52.000	50.667	49.333	42.110
29	13	Lat Thorax-muscle	Length	Right	120.000	109.000	108.667	108.333	75.317
30	13	Lat Thorax-muscle	Depth	Right	3.000	2.000	2.000	2.333	3.285
31	13	Tibia-no muscle	Width	Left	40.000	29.667	29.333	29.000	20.070
32	13	Tibia-no muscle	Length	Left	80.000	72.000	71.333	70.667	52.690
33	13	Tibia-no muscle	Depth	Left	1.000	1.000	1.000	1.000	2.400
34	13	Tibia-no muscle	Width	Right	40.000	29.333	29.333	30.000	21.030
35	13	Tibia-no muscle	Length	Right	80.000	77.000	76.000	74.333	47.600
36	13	Tibia-no muscle	Depth	Right	1.000	1.000	1.000	1.000	2.300
37	12	Neck-no muscle	Width	Left	40.000	33.000	33.333	33.000	24.380
38	12	Neck-no muscle	Length	Left	80.000	74.333	74.000	73.333	56.150

39	12	Neck-no muscle	Depth	Left	1.333	2.000	2.000	2.000	4.780
40	12	Neck-no muscle	Width	Right	40.000	30.667	30.667	31.333	23.730
41	12	Neck-no muscle	Length	Right	80.000	68.667	67.333	66.000	44.550
42	12	Neck-no muscle	Depth	Right	1.333	1.333	1.333	1.333	4.300
43	12	Lat Thorax-no muscle	Width	Left	60.000	47.000	47.000	47.000	44.770
44	12	Lat Thorax-no muscle	Length	Left	120.000	94.000	93.000	93.667	66.002
45	12	Lat Thorax-no muscle	Depth	Left	4.333	4.667	4.333	4.000	4.166
46	12	Lat Thorax-muscle	Width	Right	60.000	48.333	47.333	46.667	34.890
47	12	Lat Thorax-muscle	Length	Right	120.000	88.333	87.667	87.667	62.550
48	12	Lat Thorax-muscle	Depth	Right	5.333	4.000	4.000	4.000	4.851
49	12	Tibia-no muscle	Width	Left	40.000	26.667	26.667	26.000	20.552
50	12	Tibia-no muscle	Length	Left	80.000	73.000	72.667	71.667	53.170
51	12	Tibia-no muscle	Depth	Left	1.333	1.000	1.000	1.000	2.930
52	12	Tibia-no muscle	Width	Right	40.000	28.667	28.000	27.667	19.876
53	12	Tibia-no muscle	Length	Right	80.000	72.333	71.333	70.667	51.015
54	12	Tibia-no muscle	Depth	Right	1.000	1.000	1.000	1.000	2.050
55	11	Neck-no muscle	Width	Left	40.000	34.000	34.000	34.000	26.750
56	11	Neck-no muscle	Length	Left	80.000	78.000	77.333	76.667	56.930
57	11	Neck-no muscle	Depth	Left	2.000	2.000	2.000	2.000	2.440
58	11	Neck-no muscle	Width	Right	40.000	34.667	34.667	34.667	27.520
59	11	Neck-no muscle	Length	Right	80.000	71.333	70.667	70.667	51.000
60	11	Neck-no muscle	Depth	Right	1.667	1.333	1.000	1.333	3.320
61	11	Lat Thorax-no muscle	Width	Right	60.000	51.333	51.333	50.667	43.810
62	11	Lat Thorax-no muscle	Length	Right	120.000	112.333	111.000	110.333	82.240
63	11	Lat Thorax-no muscle	Depth	Right	2.333	2.000	2.000	2.000	2.600
64	11	Lat Thorax-muscle	Width	Left	60.000	46.667	46.333	46.333	41.420
65	11	Lat Thorax-muscle	Length	Left	120.000	97.667	97.000	96.000	73.210
66	11	Lat Thorax-muscle	Depth	Left	2.667	2.667	2.333	2.333	5.620
67	11	Tibia-no muscle	Width	Left	40.000	25.000	25.000	25.000	19.240
68	11	Tibia-no muscle	Length	Left	80.000	70.000	69.333	68.000	42.560
69	11	Tibia-no muscle	Depth	Left	1.000	1.000	1.000	1.000	2.400
70	11	Tibia-no muscle	Width	Right	40.000	28.333	29.000	29.000	27.740
71	11	Tibia-no muscle	Length	Right	80.000	72.000	71.667	71.000	41.810
72	11	Tibia-no muscle	Depth	Right	1.000	1.000	1.000	1.000	2.300
73	10	Neck-no muscle	Width	Left	40.000	36.333	36.000	36.000	29.170
74	10	Neck-no muscle	Length	Left	80.000	69.333	68.333	68.000	49.480
75	10	Neck-no muscle	Depth	Left	3.000	2.000	2.000	2.000	4.620
76	10	Neck-no muscle	Width	Right	40.000	33.000	32.667	32.333	27.130
77	10	Neck-no muscle	Length	Right	80.000	77.333	76.333	75.667	49.470
78	10	Neck-no muscle	Depth	Right	1.333	1.000	1.333	1.333	3.110
79	10	Lat Thorax-no muscle	Width	Right	60.000	54.000	54.000	53.667	44.620
80	10	Lat Thorax-no muscle	Length	Right	120.000	92.333	90.667	89.333	61.590
81	10	Lat Thorax-no muscle	Depth	Right	2.333	2.000	2.000	2.000	3.650

82	10	Lat Thorax-muscle	Width	Left	60.000	46.333	46.333	47.000	39.160
83	10	Lat Thorax-muscle	Length	Left	120.000	103.000	103.000	103.333	74.750
84	10	Lat Thorax-muscle	Depth	Left	2.667	2.000	2.000	2.000	3.700
85	10	Tibia-no muscle	Width	Left	40.000	25.667	25.667	25.000	18.470
86	10	Tibia-no muscle	Length	Left	80.000	70.000	69.667	69.333	34.730
87	10	Tibia-no muscle	Depth	Left	1.333	1.000	1.000	1.000	2.300
88	10	Tibia-no muscle	Width	Right	40.000	26.667	26.667	26.667	21.150
89	10	Tibia-no muscle	Length	Right	80.000	72.000	70.667	70.333	48.360
90	10	Tibia-no muscle	Depth	Right	1.000	1.000	1.000	1.000	2.190
91	9	Neck-no muscle	Width	Left	40.000	32.667	33.000	33.000	26.830
92	9	Neck-no muscle	Length	Left	80.000	80.000	79.667	79.000	54.960
93	9	Neck-no muscle	Depth	Left	2.000	2.000	2.000	2.000	3.000
94	9	Neck-no muscle	Width	Right	40.000	34.333	34.000	34.000	28.050
95	9	Neck-no muscle	Length	Right	80.000	79.667	78.000	76.333	55.390
96	9	Neck-no muscle	Depth	Right	2.000	2.000	2.000	2.000	2.410
97	9	Lat Thorax-no muscle	Width	Left	60.000	49.667	48.333	47.667	39.670
98	9	Lat Thorax-no muscle	Length	Left	120.000	114.333	113.667	113.000	73.980
99	9	Lat Thorax-no muscle	Depth	Left	2.333	2.000	2.000	2.333	5.700
100	9	Lat Thorax-muscle	Width	Right	60.000	47.333	47.000	46.667	40.110
101	9	Lat Thorax-muscle	Length	Right	120.000	105.333	104.333	103.000	86.370
102	9	Lat Thorax-muscle	Depth	Right	3.000	3.000	3.000	3.000	2.670
103	9	Tibia-no muscle	Width	Left	40.000	26.333	27.000	27.667	24.050
104	9	Tibia-no muscle	Length	Left	80.000	71.667	70.000	67.333	54.440
105	9	Tibia-no muscle	Depth	Left	2.000	2.000	2.000	1.667	1.840
106	9	Tibia-no muscle	Width	Right	40.000	29.667	30.333	31.333	20.750
107	9	Tibia-no muscle	Length	Right	80.000	70.000	69.000	67.667	49.570
108	9	Tibia-no muscle	Depth	Right	2.000	1.000	1.000	1.000	2.280
109	8	Neck-no muscle	Width	Left	40.000	37.333	38.000	37.667	35.340
110	8	Neck-no muscle	Length	Left	80.000	72.000	71.333	70.667	57.290
111	8	Neck-no muscle	Depth	Left	2.000	2.000	2.000	2.000	3.130
112	8	Neck-no muscle	Width	Right	40.000	34.667	34.000	33.000	29.550
113	8	Neck-no muscle	Length	Right	80.000	79.667	79.667	79.333	52.930
114	8	Neck-no muscle	Depth	Right	2.000	2.000	2.000	2.000	2.470
115	8	Lat Thorax-no muscle	Width	Left	60.000	55.333	54.000	53.333	39.070
116	8	Lat Thorax-no muscle	Length	Left	120.000	115.000	116.667	118.667	87.230
117	8	Lat Thorax-no muscle	Depth	Left	2.333	2.000	2.333	2.333	3.040
118	8	Lat Thorax-muscle	Width	Right	60.000	46.000	46.333	45.333	45.924
119	8	Lat Thorax-muscle	Length	Right	120.000	115.000	114.667	114.000	91.566
120	8	Lat Thorax-muscle	Depth	Right	3.000	3.000	3.000	2.667	2.360
121	8	Tibia-no muscle	Width	Left	40.000	28.000	27.333	27.333	20.880
122	8	Tibia-no muscle	Length	Left	80.000	73.333	74.333	73.333	52.010
123	8	Tibia-no muscle	Depth	Left	2.000	1.333	1.333	1.000	2.160
124	8	Tibia-no muscle	Width	Right	40.000	31.333	31.000	31.000	29.890
125	8	Tibia-no muscle	Length	Right	80.000	74.000	73.667	73.333	53.400
126	8	Tibia-no muscle	Depth	Right	2.000	2.000	1.667	1.667	1.700
127	7	Neck-no muscle	Width	Left	40.000	34.333	32.667	33.000	32.720

128	7	Neck-no muscle	Length	Left	80.000	78.000	72.667	70.333	56.110
129	7	Neck-no muscle	Depth	Left	2.667	2.000	2.000	2.000	3.360
130	7	Neck-no muscle	Width	Right	40.000	32.333	30.667	30.333	25.010
131	7	Neck-no muscle	Length	Right	80.000	78.333	69.000	65.667	60.550
132	7	Neck-no muscle	Depth	Right	2.667	2.333	2.333	2.333	3.220
133	7	Lat Thorax-muscle	Width	Right	60.000	48.667	45.333	44.667	42.940
134	7	Lat Thorax-muscle	Length	Right	120.000	115.000	101.333	96.333	90.760
135	7	Lat Thorax-muscle	Depth	Right	3.000	2.667	3.333	3.333	3.700
136	7	Lat Thorax- no muscle	Width	Left	60.000	48.000	45.667	44.333	44.870
137	7	Lat Thorax- no muscle	Length	Left	120.000	108.667	91.333	83.667	73.120
138	7	Lat Thorax- no muscle	Depth	Left	4.000	3.000	3.667	3.667	4.360
139	7	Tibia-no muscle	Width	Left	40.000	23.000	24.000	25.667	17.330
140	7	Tibia-no muscle	Length	Left	80.000	66.000	65.333	67.000	52.100
141	7	Tibia-no muscle	Depth	Left	2.000	2.000	1.333	1.000	2.080
142	7	Tibia-no muscle	Width	Right	40.000	33.333	24.000	21.000	13.700
143	7	Tibia-no muscle	Length	Right	80.000	78.000	66.667	62.000	46.752
144	7	Tibia-no muscle	Depth	Right	2.000	1.333	1.000	1.000	2.610
145	6	Neck-no muscle	Width	Left	40.000	37.667	37.667	37.333	26.780
146	6	Neck-no muscle	Length	Left	80.000	71.000	70.000	68.333	54.000
147	6	Neck-no muscle	Depth	Left	2.333	2.000	2.000	2.000	2.960
148	6	Neck-no muscle	Width	Right	40.000	35.000	34.000	32.667	25.810
149	6	Neck-no muscle	Length	Right	80.000	73.000	71.333	70.000	44.520
150	6	Neck-no muscle	Depth	Right	2.000	2.000	2.000	2.000	2.900
151	6	Lat Thorax-no muscle	Width	Left	60.000	55.333	53.000	51.667	47.330
152	6	Lat Thorax-no muscle	Length	Left	120.000	121.667	121.667	122.333	83.730
153	6	Lat Thorax-no muscle	Depth	Left	2.667	2.333	2.000	2.333	3.500
154	6	Lat Thorax-muscle	Width	Right	60.000	54.333	54.000	53.333	47.540
155	6	Lat Thorax-muscle	Length	Right	120.000	102.333	101.667	102.333	74.180
156	6	Lat Thorax-muscle	Depth	Right	2.667	2.333	2.333	2.333	3.360
157	6	Tibia-no muscle	Width	Left	40.000	30.000	33.000	36.000	21.911
158	6	Tibia-no muscle	Length	Left	80.000	63.333	62.667	62.333	43.830
159	6	Tibia-no muscle	Depth	Left	1.333	1.000	1.000	1.000	2.100
160	6	Tibia-no muscle	Width	Right	40.000	30.000	31.000	31.000	21.480
161	6	Tibia-no muscle	Length	Right	80.000	76.000	74.667	73.000	55.080
162	6	Tibia-no muscle	Depth	Right	1.333	1.333	1.333	1.000	2.060
163	5	Neck-no muscle	Width	Left	40.000	35.333	38.667	34.000	27.450
164	5	Neck-no muscle	Length	Left	80.000	67.333	64.667	62.000	54.550
165	5	Neck-no muscle	Depth	Left	2.333	2.000	2.000	2.000	3.050
166	5	Neck-no muscle	Width	Right	40.000	34.000	34.667	34.667	33.620
167	5	Neck-no muscle	Length	Right	80.000	66.000	64.667	59.333	61.201
168	5	Neck-no muscle	Depth	Right	2.000	2.000	2.000	2.333	4.050
169	5	Lat Thorax-no muscle	Width	Left	60.000	49.667	50.000	46.000	39.740
170	5	Lat Thorax-no muscle	Length	Left	120.000	117.667	114.333	86.667	85.980
171	5	Lat Thorax-no	Depth	Left	3.667	2.667	2.667	2.333	4.900

		muscle							
172	5	Lat Thorax-muscle	Width	Right	60.000	50.000	51.000	46.667	44.890
173	5	Lat Thorax-muscle	Length	Right	120.000	107.000	92.667	81.667	63.400
174	5	Lat Thorax-muscle	Depth	Right	3.000	3.000	3.000	3.000	4.800
175	5	Tibia-no muscle	Width	Left	40.000	29.667	32.000	26.000	22.430
176	5	Tibia-no muscle	Length	Left	80.000	70.667	70.000	60.000	49.820
177	5	Tibia-no muscle	Depth	Left	1.000	1.667	1.667	1.333	2.610
178	5	Tibia-no muscle	Width	Right	40.000	29.333	28.667	26.000	22.480
179	5	Tibia-no muscle	Length	Right	80.000	67.667	64.667	61.000	49.770
180	5	Tibia-no muscle	Depth	Right	1.000	2.000	2.000	1.000	4.250
181	4	Neck-no muscle	Width	Left	40.000	37.667	37.333	34.333	30.810
182	4	Neck-no muscle	Length	Left	80.000	71.000	63.000	61.000	45.090
183	4	Neck-no muscle	Depth	Left	2.333	2.000	2.000	1.667	2.460
184	4	Neck-no muscle	Width	Right	40.000	35.000	31.000	30.000	28.500
185	4	Neck-no muscle	Length	Right	80.000	74.667	66.667	65.000	56.630
186	4	Neck-no muscle	Depth	Right	2.000	2.000	1.667	1.333	2.400
187	4	Lat Thorax-no muscle	Width	Left	60.000	51.000	50.333	50.000	50.380
188	4	Lat Thorax-no muscle	Length	Left	120.000	122.333	105.000	96.667	102.510
189	4	Lat Thorax-no muscle	Depth	Left	2.333	2.333	2.333	2.667	2.900
190	4	Lat Thorax-muscle	Width	Right	60.000	54.333	52.667	52.667	46.770
191	4	Lat Thorax-muscle	Length	Right	120.000	102.333	89.667	86.000	83.510
192	4	Lat Thorax-muscle	Depth	Right	2.333	2.333	2.667	3.333	2.850
193	4	Tibia-no muscle	Width	Left	40.000	30.000	25.333	23.000	18.960
194	4	Tibia-no muscle	Length	Left	80.000	63.333	57.667	56.667	46.100
195	4	Tibia-no muscle	Depth	Left	1.333	1.000	1.000	1.000	2.500
196	4	Tibia-no muscle	Width	Right	40.000	30.000	28.333	27.667	28.170
197	4	Tibia-no muscle	Length	Right	80.000	75.000	67.333	66.000	46.590
198	4	Tibia-no muscle	Depth	Right	1.333	1.333	1.000	1.000	2.400
199	3	Neck-no muscle	Width	Left	40.000	35.333	34.333	33.333	29.700
200	3	Neck-no muscle	Length	Left	80.000	80.333	80.000	68.333	46.600
201	3	Neck-no muscle	Depth	Left	2.000	2.000	2.000	1.667	3.100
202	3	Neck-no muscle	Width	Right	40.000	34.667	35.000	35.000	32.900
203	3	Neck-no muscle	Length	Right	80.000	75.000	73.333	66.333	51.600
204	3	Neck-no muscle	Depth	Right	1.667	2.000	1.667	1.667	4.050
205	3	Lat Thorax-no muscle	Width	Left	60.000	52.667	51.000	46.333	46.670
206	3	Lat Thorax-no muscle	Length	Left	120.000	145.333	143.667	110.000	105.453
207	3	Lat Thorax-no muscle	Depth	Left	2.667	2.000	2.333	2.667	3.020
208	3	Lat Thorax-muscle	Width	Right	60.000	53.000	53.333	50.333	22.980
209	3	Lat Thorax-muscle	Length	Right	120.000	107.667	105.333	80.333	79.430
210	3	Lat Thorax-muscle	Depth	Right	3.333	3.000	3.333	3.333	3.400
211	3	Tibia-no muscle	Width	Left	40.000	30.667	30.667	26.000	20.600
212	3	Tibia-no muscle	Length	Left	80.000	69.333	69.000	65.333	46.430
213	3	Tibia-no muscle	Depth	Left	1.333	1.000	1.000	1.000	1.800
214	3	Tibia-no muscle	Width	Right	40.000	31.667	30.333	27.333	22.100
215	3	Tibia-no muscle	Length	Right	80.000	69.333	68.000	60.333	48.530
216	3	Tibia-no muscle	Depth	Right	1.000	1.000	1.000	1.000	2.500

APPENDIX 2

The analysis of variables by each time point

Model Information	
Data Set	WORK.ONE
Dependent Variable	_T1_T2__T2
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Least Squares Means							
Effect	**Location**	**Plane**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Location	Lat Thorax-muscle		0.1367	0.02313	210	5.91	<.0001
Location	Lat Thorax-no muscle		0.1228	0.02313	210	5.31	<.0001
Location	Neck-no muscle		0.08670	0.01636	210	5.30	<.0001
Location	Tibia-no muscle		0.1443	0.01636	210	8.82	<.0001
Plane		Depth	0.07608	0.01669	210	4.56	<.0001
Plane		Length	0.09548	0.01669	210	5.72	<.0001
Plane		Width	0.1963	0.01669	210	11.76	<.0001

Effect	Location	Plane	Location	Plane	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
Location	Lat Thorax-muscle		Neck-no muscle		0.05003	0.02833	210	1.77	0.0788	Scheffe
Location	Lat Thorax-muscle		Tibia-no muscle		-0.00754	0.02833	210	-0.27	0.7903	Scheffe
Location	Lat Thorax-no muscle		Neck-no muscle		0.03612	0.02833	210	1.27	0.2038	Scheffe
Location	Lat Thorax-no muscle		Tibia-no muscle		-0.02146	0.02833	210	-0.76	0.4496	Scheffe
Location	Neck-no muscle		Tibia-no muscle		-0.05757	0.02313	210	-2.49	0.0136	Scheffe
Plane		Depth		Length	-0.01939	0.02313	210	-0.84	0.4028	Scheffe
Plane		Depth		Width	-0.1202	0.02313	210	-5.20	<.0001	Scheffe
Plane		Length		Width	-0.1008	0.02313	210	-4.36	<.0001	Scheffe

Model Information	
Data Set	WORK.ONE
Dependent Variable	_T1_T3__T1
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Least Squares Means							
Effect	Location	Plane	Estimate	Standard Error	DF	t Value	Pr > |t|
Location	Lat Thorax-muscle		0.1411	0.02405	210	5.87	<.0001
Location	Lat Thorax-no muscle		0.1337	0.02405	210	5.56	<.0001
Location	Neck-no muscle		0.1016	0.01701	210	5.97	<.0001
Location	Tibia-no muscle		0.1710	0.01701	210	10.05	<.0001
Plane		Depth	0.08780	0.01736	210	5.06	<.0001
Plane		Length	0.1202	0.01736	210	6.92	<.0001

Location	Lat Thorax-muscle		Lat Thorax-no muscle		0.007401	0.03402	210	0.22	0.8280	Scheffe
Location	Lat Thorax-muscle		Neck-no muscle		0.03952	0.02946	210	1.34	0.1812	Scheffe
Location	Lat Thorax-muscle		Tibia-no muscle		-0.02991	0.02946	210	-1.02	0.3111	Scheffe
Location	Lat Thorax-no muscle		Neck-no muscle		0.03212	0.02946	210	1.09	0.2768	Scheffe
Location	Lat Thorax-no muscle		Tibia-no muscle		-0.03731	0.02946	210	-1.27	0.2067	Scheffe
Location	Neck-no muscle		Tibia-no muscle		-0.06944	0.02405	210	-2.89	0.0043	Scheffe
Plane		Depth		Length	-0.03239	0.02405	210	-1.35	0.1796	Scheffe
Plane		Depth		Width	-0.1147	0.02405	210	-4.77	<.0001	Scheffe
Plane		Length		Width	-0.08235	0.02405	210	-3.42	0.0007	Scheffe

Model Information	
Data Set	WORK.ONE
Dependent Variable	_T1_T4_T1
Covariance Structure	Diagonal
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Location	3	210	8.11	<.0001
Plane	2	210	15.00	<.0001

Least Squares Means							
Effect	**Location**	**Plane**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Location	Lat Thorax-muscle		0.1490	0.02086	210	7.14	<.0001
Location	Lat Thorax-no muscle		0.1485	0.02086	210	7.12	<.0001
Location	Neck-no muscle		0.1126	0.01475	210	7.63	<.0001
Location	Tibia-no muscle		0.2141	0.01475	210	14.52	<.0001
Plane		Dept	0.1031	0.01505	2	6.85	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	
Location	3	210	5.49	0.001
Plane	2	210	253.74	
		h		10		
Plane		Length	0.1484	0.01505	210	9.86	<.0001
Plane		Width	0.2165	0.01505	210	14.39	<.0001

Differences of Least Squares Means

Effect	Location	Plane	Location	Plane	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	
Location	Lat Thorax-muscle		Lat Thorax-no muscle		0.000502	0.02949	210	0.02	0.9864	Scheffe	
Location	Lat Thorax-muscle		Neck-no muscle		0.03639	0.02554	210	1.42	0.1557	Scheffe	
Location	Lat Thorax-muscle		Tibia-no muscle		-0.06509	0.02554	210	-2.55	0.0115	Scheffe	
Location	Lat Thorax-no muscle		Neck-no muscle		0.03589	0.02554	210	1.41	0.1615	Scheffe	
Location	Lat Thorax-no muscle		Tibia-no muscle		-0.06559	0.02554	210	-2.57	0.0109	Scheffe	
Location	Neck-no muscle		Tibia-no muscle		-0.1015	0.02086	210	-4.87	<.0001	Scheffe	
Plane		Depth		Length	-0.04535	0.02086	210	-2.17	0.0308	Scheffe	
Plane		Depth		Width	-0.1135	0.02086	210	-5.44	<.0001	Scheffe	
Plane		Length		Width	-0.06811	0.02086	210	-3.27	0.0013	Scheffe	

Least Squares Means

Effect	Location	Plane	Estimate	Standard Error	DF	t Value	
Location	Lat Thorax-muscle		0.3199	0.04666	210	6.86	
Location	Lat Thorax-no muscle		0.2920	0.04666	210	6.26	

Differences of Least Squares Means											
Effect	Location	Plane	Location	Plane	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	
Location	Lat Thorax-muscle		Lat Thorax-no muscle		0.02783	0.06598	210	0.42	0.6736	Scheffe	
Location	Lat Thorax-muscle		Neck-no muscle		0.1167	0.05714	210	2.04	0.0423	Scheffe	
Location	Lat Thorax-muscle		Tibia-no muscle		-0.07138	0.05714	210	-1.25	0.2130	Scheffe	
Location	Lat Thorax-no muscle		Neck-no muscle		0.08889	0.05714	210	1.56	0.1213	Scheffe	
Location	Lat Thorax-no muscle		Tibia-no muscle		-0.09922	0.05714	210	-1.74	0.0840	Scheffe	
Location	Neck-no muscle		Tibia-no muscle		-0.1881	0.04666	210	-4.03	<.0001	Scheffe	
Plane		Depth		Length	-0.8851	0.04666	210	-18.97	<.0001	Scheffe	
Plane		Depth		Width	-0.9335	0.04666	210	-20.01	<.0001	Scheffe	
Plane		Length		Width	-0.04838	0.04666	210	-1.04	0.3009	Scheffe	

APPENDIX 3

Multiple Comparisons

The ANOVA Procedure

Dependent Variable: T1 T1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	319503.2968	53250.5495	929.90	<.0001
Error	209	11968.3200	57.2647		
Corrected Total	215	331471.6168			

R-Square	Coeff Var	Root MSE	T1 Mean
0.963893	15.97120	7.567344	47.38117

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Location	3	20087.1553	6695.7184	116.93	<.0001
Plane	2	299416.0998	149708.0499	2614.32	<.0001
Side	1	0.0417	0.0417	0.00	0.9785

Dependent Variable: T2 T2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	264390.9794	44065.1632	639.99	<.0001
Error	209	14390.1682	68.8525		
Corrected Total	215	278781.1476			

R-Square	Coeff Var	Root MSE	T2 Mean
0.948382	20.02061	8.297739	41.44599

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Location	3	16888.9964	5629.6655	81.76	<.0001
Plane	2	247498.7726	123749.3863	1797.31	<.0001
Side	1	3.2104	3.2104	0.05	0.8293

The ANOVA Procedure

Dependent Variable: T3 T3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	247681.7186	41280.2864	621.57	<.0001
Error	209	13880.2438	66.4126		
Corrected Total	215	261561.9624			

R-Square	Coeff Var	Root MSE	T3 Mean
0.946933	20.15037	8.149396	40.44290

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Location	3	15614.6924	5204.8975	78.37	<.0001
Plane	2	232038.6183	116019.3092	1746.95	<.0001
Side	1	28.4079	28.4079	0.43	0.5138

Dependent Variable: T4 T4

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	228150.0535	38025.0089	645.27	<.0001
Error	209	12316.1559	58.9290		
Corrected Total	215	240466.2094			

R-Square	Coeff Var	Root MSE	T4 Mean
0.948782	19.60349	7.676521	39.15895

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Location	3	13562.9841	4520.9947	76.72	<.0001
Plane	2	214576.5504	107288.2752	1820.64	<.0001
Side	1	10.5190	10.5190	0.18	0.6731

Dependent Variable: T5 T5

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	128825.3401	21470.8900	371.53	<.0001
Error	209	12078.3033	57.7909		
Corrected Total	215	140903.6433			

R-Square	Coeff Var	Root MSE	T5 Mean
0.914280	24.33043	7.602034	31.24496

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Location	3	11702.4845	3900.8282	67.50	<.0001
Plane	2	117122.3345	58561.1672	1013.33	<.0001
Side	1	0.5211	0.5211	0.01	0.9244

The ANOVA Procedure
Repeated Measures Analysis of Variance
Tests of Hypotheses for Between Subjects Effects

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Locati	3	76631.039	25543.680	98.35	<.00

on					01
Plane	2	1086844.405	543422.202	2092.25	<.0001
Side	1	25.497	25.497	0.10	0.7544
Error	209	54283.671	259.730		

The ANOVA Procedure
Repeated Measures Analysis of Variance
Univariate Tests of Hypotheses for Within Subject Effects

Source	DF	Anova SS	Mean Square	F Value	Pr > F	Adj Pr > F	
						G - G	H-F-L
time	4	28966.81261	7241.70315	584.96	<.0001	<.0001	<.0001
time*Location	12	1225.27390	102.10616	8.25	<.0001	<.0001	<.0001
time*Plane	8	23807.97079	2975.99635	240.39	<.0001	<.0001	<.0001
time*Side	4	17.20299	4.30075	0.35	0.8459	0.7944	0.7977
Error(time)	836	10349.52019	12.37981				

APPENDIX 4

Geometric Least Means Square Procedure

The GLM Procedure

Scheffe's Test for T1

Alpha	0.05
Error Degrees of Freedom	210
Error Mean Square	56.9922
Critical Value of F	2.64760

Comparisons significant at the 0.05 level are indicated by *.**				
Location Comparison	**Difference Between Means**	**Simultaneous 95% Confidence Limits**		
Lat Thorax-muscle - Lat Thorax-no muscle	0.056	-4.959	5.070	
Lat Thorax-muscle - Neck-no muscle	20.380	16.037	24.723	***
Lat Thorax-muscle - Tibia-no muscle	20.588	16.245	24.931	***
Lat Thorax-no muscle - Lat Thorax-muscle	-0.056	-5.070	4.959	
Lat Thorax-no muscle - Neck-no muscle	20.324	15.981	24.667	***
Lat Thorax-no muscle - Tibia-no muscle	20.532	16.189	24.875	***
Neck-no muscle - Lat Thorax-muscle	-20.380	-24.723	-16.037	***
Neck-no muscle - Lat Thorax-no muscle	-20.324	-24.667	-15.981	***
Neck-no muscle - Tibia-no muscle	0.208	-3.338	3.754	
Tibia-no muscle - Lat Thorax-muscle	-20.588	-24.931	-16.245	***
Tibia-no muscle - Lat Thorax-no muscle	-20.532	-24.875	-16.189	***
Tibia-no muscle - Neck-no muscle	-0.208	-3.754	3.338	

The GLM Procedure

Scheffe's Test for T2

Alpha	0.05
Error Degrees of Freedom	210
Error Mean Square	68.5399
Critical Value of F	2.64760

Comparisons significant at the 0.05 level are indicated by *.**			
Location Comparison	**Difference Between Means**	**Simultaneous 95% Confidence Limits**	

Lat Thorax-no muscle - Lat Thorax-muscle	3.472	-2.027	8.972	
Lat Thorax-no muscle - Neck-no muscle	18.593	13.830	23.355	***
Lat Thorax-no muscle - Tibia-no muscle	21.750	16.987	26.513	***
Lat Thorax-muscle - Lat Thorax-no muscle	-3.472	-8.972	2.027	
Lat Thorax-muscle - Neck-no muscle	15.120	10.358	19.883	***
Lat Thorax-muscle - Tibia-no muscle	18.278	13.515	23.040	***
Neck-no muscle - Lat Thorax-no muscle	-18.593	-23.355	-13.830	***
Neck-no muscle - Lat Thorax-muscle	-15.120	-19.883	-10.358	***
Neck-no muscle - Tibia-no muscle	3.157	-0.731	7.046	
Tibia-no muscle - Lat Thorax-no muscle	-21.750	-26.513	-16.987	***
Tibia-no muscle - Lat Thorax-muscle	-18.278	-23.040	-13.515	***
Tibia-no muscle - Neck-no muscle	-3.157	-7.046	0.731	

The GLM Procedure

Scheffe's Test for T3

Alpha	0.05
Error Degrees of Freedom	210
Error Mean Square	66.23167
Critical Value of F	2.64760

Comparisons significant at the 0.05 level are indicated by ***.				
Location Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Lat Thorax-no muscle - Lat Thorax-muscle	3.426	-1.980	8.832	
Lat Thorax-no muscle - Neck-no muscle	17.870	13.189	22.552	***
Lat Thorax-no muscle - Tibia-no muscle	20.977	16.295	25.659	***
Lat Thorax-muscle - Lat Thorax-no muscle	-3.426	-8.832	1.980	
Lat Thorax-muscle - Neck-no muscle	14.444	9.763	19.126	***
Lat Thorax-muscle - Tibia-no muscle	17.551	12.869	22.233	***
Neck-no muscle - Lat Thorax-no muscle	-17.870	-22.552	-13.189	***
Neck-no muscle - Lat Thorax-muscle	-14.444	-19.126	-9.763	***
Neck-no muscle - Tibia-no muscle	3.106	-0.716	6.929	
Tibia-no muscle - Lat Thorax-no muscle	-20.977	-25.659	-16.295	***
Tibia-no muscle - Lat Thorax-muscle	-17.551	-22.233	-12.869	***
Tibia-no muscle - Neck-no muscle	-3.106	-6.929	0.716	

The GLM Procedure

Scheffe's Test for T4

Alpha	0.05
Error Degrees of Freedom	210
Error Mean Square	58.6984

	5
Critical Value of F	2.64760

Comparisons significant at the 0.05 level are	
Location Comparison	**Difference Between Means**
Lat Thorax-no muscle - Lat Thorax-muscle	2.435
Lat Thorax-no muscle - Neck-no muscle	16.157
Lat Thorax-no muscle - Tibia-no muscle	19.315
Lat Thorax-muscle - Lat Thorax-no muscle	-2.435
Lat Thorax-muscle - Neck-no muscle	13.722
Lat Thorax-muscle - Tibia-no muscle	16.880
Neck-no muscle - Lat Thorax-no muscle	-16.157
Neck-no muscle - Lat Thorax-muscle	-13.722
Neck-no muscle - Tibia-no muscle	3.157
Tibia-no muscle - Lat Thorax-no muscle	-19.315
Tibia-no muscle - Lat Thorax-muscle	-16.880
Tibia-no muscle - Neck-no muscle	-3.157

The GLM Procedure

Scheffe's Test for T5

Alpha	0.05
Error Degrees of Freedom	210
Error Mean Square	57.51821
Critical Value of F	2.64760

Comparisons significant at the 0.05 level are indicated by ***.				
Location Comparison	**Difference Between Means**	**Simultaneous 95% Confidence Limits**		
Lat Thorax-no muscle - Lat Thorax-muscle	1.486	-3.552	6.524	
Lat Thorax-no muscle - Neck-no muscle	13.918	9.555	18.281	***
Lat Thorax-no muscle - Tibia-no muscle	17.951	13.588	22.314	***
Lat Thorax-muscle - Lat Thorax-no muscle	-1.486	-6.524	3.552	
Lat Thorax-muscle - Neck-no muscle	12.432	8.069	16.795	***
Lat Thorax-muscle - Tibia-no muscle	16.465	12.102	20.828	***
Neck-no muscle - Lat Thorax-no muscle	-13.918	-18.281	-9.555	***
Neck-no muscle - Lat Thorax-muscle	-12.432	-16.795	-8.069	***
Neck-no muscle - Tibia-no muscle	4.033	0.470	7.595	***
Tibia-no muscle - Lat Thorax-no muscle	-17.951	-22.314	-13.588	***
Tibia-no muscle - Lat Thorax-muscle	-16.465	-20.828	-12.102	***
Tibia-no muscle - Neck-no muscle	-4.033	-7.595	-0.470	***