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Phonatory Characteristics of the Excised Human Larynx in Comparison to other Species

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Abstract

Objective—The purpose of this study was to (a) determine the conditions needed to elicit phonation from excised human larynges and the resultant range of phonations produced, (b) compare that with similar information previously obtained from canine, pig, sheep and cow and (c) relate those findings to previously reported information about viscoelastic properties of the vocal fold tissue (i.e. stress strain curves and Young's Modulus).

Methods—Six human larynges of the geriatric group (age ranges 70–89) were mounted on the bench without supraglottic structures and phonation was achieved with the flow of heated and humidified air through the tracheal tube. Using various sutures to mimic the function of the laryngeal muscles, the larynges were put through a series of sustained oscillations with adduction as a control parameter.

Results—The human larynges oscillated with an average frequency that was close to the canine larynges, but the oscillation behavior and wide frequency range were similar to those of pig larynges. The similarity of the wide vibration frequency ranges of human and pig larynges may be due to the nonlinear behavior of their elasticity, which is related to their vocal folds high collagen content. On the contrary, other species with limited frequency ranges showed almost linear stress-strain curves due to the higher elastin and lower collagen contents.

Conclusions—The physiological differences in the linearity and ranges of oscillation of excised larynges reported in this study and previous studies are reflective of the tissue composition and mechanics.

Keywords

Excised human larynx; fundamental frequency; nonlinear stress-strain curves; collagen and elastin

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INTRODUCTION

Vibratory characteristics of vocal folds, including its frequency and amplitude, are dependent upon many physiological, biomechanical, and aerodynamic factors that vary across species and within individual samples. For example, vocal fold tissue morphology and tissue elasticity are important determinants of resultant biomechanical properties such as tension and elongation. Additionally, subtle changes in frequency and amplitude are controlled by the contractile properties of intrinsic laryngeal muscles.¹ Because vibration of the vocal folds is induced by and closely coupled with the glottal airflow, the vibratory behavior is dependent on the glottal aerodynamics as well. The primary control factors for vocal pitch at the glottal source include vocal fold tension, vocal fold elongation, and subglottal air pressure.^{2,3} The tension in the vocal folds may be further classified as active, passive, or a combination of both. The active tension in the vocal folds results from thyroarytenoid (TA) muscle contraction and the passive tension results from cricothyroid (CT) muscle contraction with the resultant increase in vocal fold length and stretching of the mucosa. In reality a combination of active and passive tensions typically results from co-contraction of CT and TA muscles.⁴ In the vocal folds, the increase in length and tension are not independent of each other and the relationship between the two is determined by the tissue elasticity. The tissue elasticity also determines the relationship between the increased amplitude of vibration (and resultant increase in longitudinal tension of the vocal folds) with an increase in glottal pressure and frequency.

Several studies have examined vocal behavior based on vocal fold morphology and histology,^{5,6} measurement of the vocal fold elastic properties,⁷ X-ray and lamina-graphic studies of vocal folds,⁸ and excised larynx studies using human or animal larynges.^{9,10} Morphological and histological studies of the vocal folds² have helped reveal the complex tissue structure of the vocal folds and studies of excised human and animal larynges have enhanced our understanding of the glottal aerodynamics and vocal function.

Cross-species studies of excised larynges, using an *in vitro* setup, have made major contributions in our understanding of the vibratory characteristics of the vocal folds. For example, phonatory data from different species using the excised larynx model have demonstrated that fundamental frequency is strongly dependent on subglottal pressure³ and that different species not only have different ranges of fundamental frequency, but also have different frequency-pressure behaviors,¹⁰ which could be related to differences in their vocal fold tissue morphology. These differences in the oscillation frequency range should theoretically correlate with their vocal fold lengths, stiffness profiles, and histological makeup. So, to understand the vocal vibratory behavior, not only knowledge of vocal fold geometry and morphology is needed, but it is equally important to examine the contribution of individual components of the vocal folds and their elastic behavior to the overall physiology.

Histological works by Hirano¹¹ described the stratified structure of the human vocal folds, which were differentiated as epithelium, lamina propria, and vocalis muscle. The lamina propria was further divided into three sub-layers based on differing concentrations of elastin and collagen, the fibrous proteins that determine the elasticity and tensile strength of these tissues respectively. Lamina propria (LP) was composed of the superficial layer (SLLP), characterized as loose and pliable, the intermediate layer (ILLP), consisting of mainly elastin fibers and the deep layer (DLLP), consisting of mainly collagenous fibers.¹² The ILLP and the DLLP together constitute the vocal ligament, an area of the true vocal folds that bears the longitudinal stress and has a higher concentration of collagen fibers. In addition, the extracellular matrix is also composed of interstitial proteins like hyaluronic acid that provide the viscosity to the tissue. Thus, the vocal folds present a varied

histological profile, which would be expected to be reflected in the viscoelastic properties of the tissues.

Gray et al¹³ described the histological make-up of the human vocal fold tissue and related it to the biomechanical properties of the fibrous proteins. On application of stress to the excised human vocal ligament fibers, the researchers observed that the stress-strain curve was linear for low stress levels but became nonlinear at higher levels.¹⁴ It was speculated that the elastin fibers were recruited for the linear part and the collagen for the non-linear steep section of the curve reflecting its role in pitch control by increasing stiffness rather than length. Kurita et al⁶ did a cross-species comparison of the thickness of the mucosa and the density of collagen and elastic fibers in the lamina propria of the vocal folds. The vocal folds from larynges of dog, pig, sheep, ox and human were sectioned, stained and examined under a microscope. Besides the difference in the vocal fold membranous length and mucosal thickness, there were significant differences in the elastin and collagen contents of these species. For example, dog vocal folds had a thick mucosal layer (3 mm) with a high concentration of elastin and collagen in the superficial layer. The sheep vocal folds were similar in length and thickness to human, but with two layers with fairly abundant elastic and collagenous fibers. In contrast to the dog and sheep vocal folds, the pig larynx demonstrated a human-like superficial layer with sparse fibrous components and increasing collagenous fiber and decreasing elastic fiber content close to the vocalis muscle. Garrett et al¹⁵ compared the histological structure of dogs, monkeys, and pigs with human vocal fold tissue and observed a 3-layered rather than 2-layered structure as documented by Kurita et al.⁶ They also reported two less distinct layers in pig vocal folds, with the SLLP composed of mostly ground substance and the DLLP consisting of mostly collagen and minimal elastin fibers.

These studies suggest major differences in the tissue structure of vocal folds across species and differences in their vibratory behavior. The purpose of this study was to obtain phonatory characteristics of excised human larynges and compare it with those of other available excised animal models such dog, pig, sheep and cow for a better understanding of the mechanism of pitch control through correlating of the vibratory behavior of vocal fold tissue of different species to their tissue elasticity. To achieve this, besides obtaining aerodynamic and acoustic data from human excised larynges, the elastic and aerodynamic properties of other species such as dog, pig, sheep, and cow were included from our previous studies and compared with those of human larynges.

METHODS

The experimental protocol for the excised human larynges was similar to that of previous work^{9,16} and will be described here briefly. Six excised human larynges from a geriatric group, with ages ranging from 70–89 years (see Table 1), were thawed overnight in saline solution and cleaned with removal of the extraneous tissues and supraglottic structures, including the epiglottis and false vocal folds. Then, electrode plates from a Synchrovoice electroglottograph (EGG) were placed on either side of the thyroid lamina to obtain the EGG signal during phonation to determine fundamental frequency (see Figure 1 for laryngeal prep). The prepared larynx was mounted on appropriately sized PVC tubing, with an adaptor for attaching it to the excised bench air tube.

Air from the building pipeline passed through an in-line filter and a flow meter (Gilmont rotameter, model J197) for air flow rate monitoring. The air was then heated and humidified to about 37°C and 100% humidity (ConchaTherm III, Hudson RCI) before entering the mounted larynx. The experimenters wore gloves, goggles, and breathing masks for protection against any possible pathogens from the specimens. A pressure tap located about

10 cm below the larynx, was used to monitor the subglottal pressure through a well-type manometer (Dwyer Model 1230-8). The sound pressure level was measured with a sound level meter (Extec model 407738) placed about 15 cm from the larynx.

Adduction was controlled by a pair of sutures pulling on the muscular process of each arytenoid cartilage to simulate lateral cricoarytenoid and thyroarytenoid (TA) muscle activity, as in arytenoid adduction. The adduction levels of low, medium, and high were adjusted using weights (50–150 grams) that pulled the sutures that were attached to the muscular processes of the arytenoid cartilages.

Each excised larynx experiment started with two pressure-flow sweeps at all three levels of vocal fold adduction to cover the variety of oscillatory conditions. However, adduction was not a control parameter in this study. The flow rate in the sweep was gradually increased or decreased with a rotary control valve (consequently altering pressure as well) to determine the range of aerodynamic conditions during which the vocal folds would vibrate. Then, a series of sustained phonations were performed, within the working range of pressure and flow for every adduction level, to record and observe oscillation of the vocal folds. In each pressure-flow sweep, flow rate was increased gradually until oscillation started and then continued until either subglottal pressure or flow rate reached the maximum allowable limit (usually 30 cm H₂O for pressure and 1.4 L/s for flow rate).

Analog signals from the EGG, microphone, pressure, and flow transducers were recorded simultaneously on a Sony SIR1000 digital tape recorder at a sampling rate of 40,000 samples per second for each channel and digitized onto a computer using a DATAQ A/D converter and WINDAQ software. The recorded signals were then converted to physical values with MATLAB software routines and used for the aerodynamic and acoustic analysis. Mean values of flow rate, subglottal pressure, phonation threshold pressure, pressure amplitude, and fundamental frequency were calculated during each sustained oscillation and during each pressure-flow sweep.

To calculate the fundamental frequency, the EGG signal was low-pass filtered at 150% of its estimated F₀ value seen from the spectrogram or an oscilloscope. The fundamental frequency was then calculated with a zero crossing method. First, the signal DC offset was removed and then the periods of all the cycles in the selected segment were calculated from consecutive zero crossings and averaged.

Each pressure-flow sweep was divided into 60–100 segments such that each segment included at least 10 cycles from which mean values of frequency could be calculated. Sound pressure level (SPL) during each sweep was also calculated from the microphone signal. This was accomplished with SPL readings during sustained phonations and by finding calibration coefficients by fitting SPL values to RMS values of the microphone signals. A second order polynomial was fitted to 8–10 data points (sustained oscillation values) for this purpose. With these calculations, each sweep could be regarded as a series of 60–100 consecutive oscillations from which we could observe the oscillation behavior of the larynx; also, we could estimate its glottal flow resistance and slope of frequency increase with subglottal pressure (dF/dP).

Although, dog vocal folds elastic properties were available from Alipour and Titze⁷ for viable tissue samples, we have repeated stress-strain experiment for five samples of the nonviable dog vocal folds with set up similar to human tissues, to have a compatible data set for comparison. First, frozen canine larynges were thawed within the saline solution in the refrigerator overnight. Then, two samples were made from each canine vocal fold's cover with attached cartilages at each end. Samples were mounted in an ergometer and were

subjected to 10 cycles of slow (1 Hz) stretch and release. The stress-strain curve and low strain Young's modulus were obtained similar to those of Alipour and Titze⁷.

Statistical analysis was performed using Microsoft Excel 2010 to determine if there were significant differences between phonatory conditions across species. Unpaired t-tests with the significance level of $p=0.05$ were performed to determine if there were significant differences between individual phonatory and viscoelastic characteristics.

RESULTS

One aim of this study was to determine the conditions needed to elicit phonation (i.e. phonation threshold pressure and subglottic pressure) from the excised human larynges and the resultant range of phonations produced (i.e. F0 and SPL). Figure 2 provides a representative sample of data obtained during one pressure-flow sweep. It shows five curves that are the mean values for excised human larynx #4 in an upward pressure-flow sweep as a function of time. The curves are, from top to bottom, subglottal pressure, flow rate, fundamental frequency, subglottal AC pressure or pressure amplitude (one half of the peak-to-peak pressure), and sound pressure level (estimated from the microphone signal) respectively. After two seconds from the beginning of oscillations, the increase in flow was accompanied by a nearly linear increase in pressure and in frequency. While pressure and flow increased monotonically, at around 6.4 second (around the notch on the pressure and flow curves), the fundamental frequency suddenly raised to another level (about 15 Hz), however with a similar increasing slope of 5.9 Hz/ cm H₂O. Interestingly, this event causes the pressure amplitude to drop to a minimum around 13 seconds and then rise. Video observations of this larynx from a superior view suggested an increase of the glottal opening after 6.4 seconds, which can be observed from EGG signal. The sound pressure level has an increasing trend until 13 seconds, and then slows down its ascent until the end of the sweep, which shows more increase.

Figure 3 combines the oscillatory conditions of the six excised human larynges. This includes average phonation threshold pressure (PTP), subglottal pressure (Ps), sound pressure level (SPL), and fundamental frequency (F0), with the values indicated at the top of each column. The sound pressure level was not recorded for larynges 1–3 due to equipment failure. While each larynx oscillated at a different range of pressures, flow rates (not shown here) and frequencies, the excised human larynx oscillated at low pressure ranges. Due to the advanced age of the subjects, the larynges included large amounts of fatty tissue and their oscillations were characterized by high jitter, which made the data analysis challenging. Most excised larynges started to oscillate at PTP ranges of 2–5 cm H₂O. The ranges of Ps, PTP, flow rate and fundamental frequency are included in Table 1.

A second aim of the study was to compare the phonatory characteristics of human excised larynges with other animal excised models. We adopted published phonatory data for the pig, sheep, and cow larynges from Alipour and Jaiswal¹⁰ and for the canine larynges from Alipour and Scherer,³ for oscillating conditions with adduction controlled and with no vocal fold elongation in all excised larynges. Although there was no initial elongation of the vocal folds, the dynamic strains during oscillations could play an important role in the vibratory characteristics.

A comparison chart of phonatory conditions across these species and human is presented in Figure 4. Here the average values of phonation threshold pressure (PTP), subglottal pressure (Ps), sound pressure level (SPL), and fundamental frequency (F0) are compared. The average F0 of human appears to be similar to that of canine ($p=0.004$). The average SPL value of the human excised larynx was close to those of dog ($p=0.08$) and cow ($p=0.08$), but

larger than the average value of sheep ($p=0.003$) and smaller than the average value of pig ($p=1.6 \times 10^{-10}$). The human PTP average was close the cow larynges ($p=0.32$), but smaller than those of canine ($p=0.01$), sheep ($p=4.5 \times 10^{-5}$), and pig ($p=6 \times 10^{-8}$). The mean values of human excised subglottal pressure was close to the mean value of dog ($p=0.49$) but smaller than cow ($p=0.0004$), sheep ($p=0.003$), and pig ($p=1.3 \times 10^{-7}$). Thus, the human excised larynx operates at similar subglottal pressure and frequency as the canine excised larynx.

The similarity of human fundamental frequency to canine provides an incentive to look at the phonatory characteristics of canine for better comparison. Figure 5 shows such data for 7 excised canine larynges collected in a similar fashion. This data suggests that canine larynges oscillate at frequencies that are in the range of a male human, PTP values that are a little higher than human corresponding values, subglottal pressures in higher ranges, and similar sound pressure levels.

However, the most important aspect of the phonatory characteristics is the fundamental frequency, its range, and its behavior during subglottal pressure changes. To understand the frequency behavior of the human excised larynx in comparison to other species, the typical frequency pressure behavior of these species is depicted in Figure 6. Here, changes of F_0 with P_s are shown for all species during the pressure-flow sweep, where pressure and flow were gradually increased and fundamental frequency data were obtained through EGG analysis of every 10–20 cycles of oscillation. The cow vocal fold data are at the bottom of the graph (represented by diamonds) with the lowest frequencies corresponding to its greater length (about 27 mm) and a linear graph with small slope (0.75 Hz/cm- H_2O). The sheep vocal folds (circles) oscillate between 80–100 Hz for pressures below 16 cm- H_2O , with a slope of 1.4 Hz/cm- H_2O . Dog vocal folds (squares) have a larger slope of 2.1 Hz/cm- H_2O . The widest range of frequency (150–400 Hz) is observed in the pig larynx (hollow triangles), with a large slope of 13.7 Hz/cm- H_2O . The human larynx (filled delta) has the highest slope next to the pig, with average slope of 6 Hz/cm- H_2O . Thus, a similarity of range and slope between human and pig larynges exist that has roots in their vocal folds micro structures and their elastic behaviors. On average these slopes are 6.0 ± 3.7 Hz/cm- H_2O for pig¹⁰, 2.9 ± 0.7 Hz/cm- H_2O for dog³ and 4.7 ± 2.4 Hz/cm- H_2O for the human samples of this study.

A third aim was to relate our findings to previously reported information about viscoelastic properties of the vocal fold tissue (i.e. stress-strain curves and Young's Modulus). Elastic data for the pig, sheep, and cow were adopted from Alipour et al¹⁷ and for human data from Alipour and Vigmostad.¹⁸ The canine data was new data collected for this study, as indicated in the methodology. In Figure 7, the average low strain Young's modulus (strain < 15) for dog, pig, sheep, cow, and human are represented by bar graphs with the error bars representing the standard deviations. The vocal fold stiffness or Young's modulus is highest for cow (31.6 ± 8.1 kPa), then human (30.0 ± 13.4 kPa), followed by dog (19.9 ± 10.1 kPa), pig (19.7 ± 4.8 kPa), and the sheep (12.0 ± 2.7 kPa) is the lowest. Thus, each species has vocal folds of different length, thickness, and stiffness.

Figure 8 shows the elastic behaviors of typical vocal fold cover samples from the dog, cow, pig, and human. The sheep larynx, which did not show any similarity, was left out. The solid line in each loop represents the stretch and the dashed line the release phase. The Young's modulus or degrees of tissue stiffness can be determined from the slope of the stretch phase of these loops. Each stretch curve starts with a smaller slope and ends with a larger slope, suggesting nonlinearity of their elasticity. Thus, there are two Young's modulus values for each sample, a low strain value (E0) for strains less than 0.15 and a high strain value (EH) for the last 0.05 value of strain. The figure shows that cow vocal fold tissue, with the lowest EH/E0 ratio of 2, is the least nonlinear followed by dog with an EH/E0 ratio of 3. The

human vocal fold tissue, with a ratio of about 16, is the most nonlinear sample. The pig vocal fold ($EH/E0 = 13.5$) was most similar in elastic behavior to the humans.

DISCUSSION

Figure 3 and Table 1 showed that excised human larynges oscillated with PTP ranges of 2–5 cm H₂O. This is consistent with PTP ranges reported by Mau et al¹⁹ for their offset PTP. The ranges of P_s , flow rate, and fundamental frequencies are also included in the table. The range of frequency is similar to what was reported by van den Berg and Tan⁹ in their excised human results for chest voice. The range of 4–28 cm H₂O for subglottal pressure is also consistent with those of van den Berg and Tan⁹. However, the range of flow rates is higher in this study, which could be due to the different adduction control mechanisms.

To compare human phonatory behavior to other species, we need to consider their differences in vocal structure. Vocal folds contain two primary load-bearing proteins responsible for providing elasticity (elastin) and strength (collagen). Collagen is a wavy, fibrous protein and in the vocal folds is arranged in a complex 3-D network. During stretching of the tissue, the wavy collagen becomes taut initially and the tension in the tissue increases as additional collagen fibers are recruited. Different species have different distributions of these proteins in their layered structure. Thus, we should expect different elastic behavior and different vibratory characteristics.

Figure 7 showed comparative elasticity of different species, where we noticed that similarity exists between dog and pig and also between cow and human. So, it is not the value of Young's modulus that results in phonation similarity. The stress-strain curves shown in Figure 8 demonstrate the nonlinear elastic behavior of the soft tissue. There is a fairly abrupt change as elongation increases past 0.2. The nonlinearity is likely a result of the collagen content in the tissue, where collagen recruitment occurs gradually as the sample is stretched. For small strains (<0.15), the stress-strain relationship can be assumed to be linear, with the slope of the curve depicting a low Young's modulus value for the sample. In this low-strain linear region of the curve, it is reasonable to assume that elastin is the primary load-bearing component within the tissue. Upon further displacement, initially unstretched collagen fibers are recruited and begin to bear load. In this way, Young's modulus for the elastin content in the tissue may be assumed by the linear region of the graph.

The variability of data in Figure 4 could be in part related to the variability in elastin and collagen content of the tissue and in part attributed to the small number of samples. Several groups have shown that both age and gender affect elastin and collagen content, which in turn would influence the material properties of the tissue. As can be observed in the stress-strain curves, the linear strain region appears to vary between samples. Beyond the linear region, the strength and density of primarily the collagen fibers will affect the stress-strain relationship in the non-linear section.

The wide ranges of oscillation frequency in the human and pig larynges can be attributed to their nonlinear stress-strain curves. As Kurita et al² reported, there is good similarity between human and pig vocal fold histology and our elastic data confirmed this fact because of the relation of the structure and function of the tissues. Similarity exists in the range of pressure-frequency slopes (dF/dP), with mean values of 4.7 and 6.0 Hz/cm H₂O for the human and pig larynges. On the other hand, cow larynges, which have very stiff vocal folds, do not have much frequency range. This could be due to the increased amount of elastin and decreased amount of collagen, which resulted in a more linear elastic behavior. Studies of histological and phonatory properties of different species indicated relations between phonation and structure.

CONCLUSIONS

This study provides a biomechanical and phonatory comparison of the vibrating tissue samples across four different species. The results suggest that physiological differences in the linearity and ranges of oscillation of excised larynges are reflective of the tissue composition and mechanics. It was found that the canine larynx oscillates very similar to the human excised larynx, but the two do not have similar pressure- frequency behavior. Also, nonlinearity of the stress-strain curves for vocal fold tissues is responsible for the wide range of frequencies in human and pig larynges. Histological examination of the vibrating tissue across these species would aid in further parsing out the effect of makeup and distribution of fibrous composition on the differences in the tissue material properties between species. In other words, the differences in the phonatory function among these species could have attributed, not only to their geometries, but also to their molecular composition and the stiffness characteristics of the vocal folds as well.

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Figure 1.
A mounted excised human larynx with control sutures and EGG electrodes.

Figure 2. Moving averaged values of subglottal pressure (P_s), flow rate (Fl), fundamental frequency (F_0), pressure amplitude (P_a), and sound pressure level (SPL) for excised human larynx # 4 during pressure-flow increasing sweep.

Figure 3.

Averaged values of phonation threshold pressure (PTP), subglottal pressure (Ps), sound pressure level (SPL), and fundamental frequency (F0) across 6 human excised larynges, with average values and standard deviation on top of each bar. Due to the equipment failure, the SPL is missing for the larynges 1–3.

Figure 4. Comparative phonatory characteristics of pig, sheep, cow, dog, and human larynges, including phonation threshold pressure (PTP), subglottal pressure (Ps), sound pressure level (SPL), and fundamental frequency (F0).

Figure 5.

Phonatory characteristics of canine larynges including average values of the phonation threshold pressure (PTP), subglottal pressure (Ps), sound pressure level (SPL), and fundamental frequency (F0).

Figure 6. Pressure-frequency relations for typical samples of dog, pig, sheep, cow, and human excised larynges during increasing pressure-flow sweeps.

Figure 7.

Average Young's modulus comparison of vocal fold samples from different species. Each bar represents a species with its standard deviation as error bar.

Figure 8.

Stress-strain loops of various vocal fold samples. A- dog vocal folds, B- cow vocal folds, C- pig vocal folds, and D- human vocal folds. E0 stands for low-strain Young's modulus and EH for high-strain Young's modulus (both in kPa).

Table 1

Phonatory characteristics of the excised human larynges including their gender, age and oscillating ranges. Ps stands for the subglottal air pressure, PTP for phonation threshold pressure, and F0 for fundamental frequency.

Larynx	Gender	Age	Ps (cm H ₂ O)	PTP (cm H ₂ O)	Flow rate (ml/s)	F0 (Hz)
1	Male	89	4 -- 25	2 -- 4	175 -- 1400	79.6 -- 193.4
2	Female	75	6 -- 20	5 -- 10	410 -- 1400	77.1 -- 285.3
3	Male	70	4 -- 16	3 -- 3	490 -- 1390	106.8 -- 215.2
4	Male	77	6 -- 24	4 -- 6	340 -- 1400	89.5 -- 166.7
5	Male	75	6 -- 28	4 -- 5	215 -- 1400	88.3 -- 361.4
6	Male	75	4 -- 19	2 -- 3	175 -- 590	97.3 -- 152.7
Mean		76.8	13.3	4.2	760	153.4