Biological Mechanisms Underlying Voice Changes Due to Dehydration

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Four vocally untrained healthy adults, 2 men and 2 women, completed the study. A double-blind placebo-controlled approach was used to administer three treatments to each participant on separate days. Drugs treatments involved a single 60-mg dose of a diuretic, Lasix (LA), on one day, and a single 50-mg dose of an oral antihistamine, diphenhydramine hydrochloride (DH), on another day. A third day involved the administration of a placebo, sugar pills (SP). Critical posttreatment measures were weight (kg), which estimated systemic dehydration, saliva viscosity (centipoise), which estimated secretion dehydration, and phonation threshold pressure (PTP, in cm H_2O), at high pitches, which indicated pulmonary drive for phonation. The central experimental question was: Does systemic dehydration, or secretory dehydration, or both, mediate increases in PTP that are known to occur following dehydration treatments? The results showed that LA induced systemic dehydration, as shown by a decrease in total body mass of about 1%. Weight losses were seen during a 1- to 4-hour block following drug administration and persisted for at least 8 hours thereafter. PTPs also increased in that condition, about 23% relative to baseline, but only several hours *after* whole-body dehydration was initially seen (5–12 hours after drug administration). In contrast, no evidence was seen that DH accomplished either secretory dehydration or PTP shifts. The results indicate that systemic dehydration can mediate PTP increases. The influence of secretory dehydration on PTP is unclear.

KEY WORDS: voice, dehydration, viscosity, phonation threshold pressure

Hydration has long been a source of interest in basic and clinical voice science. In this study, we address questions about the mechanisms by which dehydration produces known changes in the subglottic pressure required for vocal fold oscillation. As background to the specific experimental questions, we begin with a review of previous studies and questions that they raised.

Several experimental reports have focused on how hydration influences vocal fold vibration mechanics. Two studies of healthy adults explored the combined effect of air humidity, water consumption, and drugs on phonation threshold pressure (PTP), the minimum subglottic pressure required to initiate and sustain vocal fold oscillation (Verdolini, Titze, & Fennell, 1994; Verdolini-Marston, Titze, & Druker, 1990). In both studies, the results revealed an inverse relation between hydration treatment and PTP. Effects were particularly robust at high pitches (see also Verdolini-Marston, Sandage, & Titze, 1994). Parallel results have been seen in excised canine larynx studies in which hydration variably has been manipulated by osmotic gradients across the mucosa and by the direct delivery of dessicated air to induce phonation (Finkelhor, Titze, & Durham, 1988; Jiang, Verdolini, Ng, & Hanson, 2000).

The proposed theoretical basis for the findings has to do with vocal fold tissue viscosity. Viscosity is a measure of resistance to flow. The viscosity of pure water is 0.01 poise (P). Average viscosity of vocal fold tissue is about 0–10 P, in comparison to the viscosity of surface tissues, which is closer to 100 P (Titze & Talkin, 1979). According to a theory of small-amplitude oscillations in barely abducted vocal folds, as in phonation threshold events (Titze, 1988), the following relations, for a rectangular glottis, are indicated:

$$P_L \geq \frac{2k}{T} \times Bc \times \frac{w}{2}$$

where k is a dimensionless constant representing a transglottal pressure coefficient (about 1.1); T is vocal fold thickness; B is a tissue damping coefficient, which is a lumped-element representation of tissue viscosity; c is the velocity of mucosal wave propagation on the tissue surface; and $w/2$ is the prephonatory glottal half-width at the vocal processes of the arytenoids. The equality in the equation represents PTP, or the minimum subglottic pressure required for vocal fold oscillation. With relevance to the present discussion, increases in tissue viscosity B are predicted to increase PTP, and decreases in tissue viscosity are predicted to decrease it.

A key question regards how vocal fold viscosity may be manipulated in human participants. If water can be added and subtracted from vocal fold tissue, the tissue's viscosity should be respectively decreased or increased, theoretically producing parallel changes in PTP. The experimental results described above are consistent with the idea that the de/hydration treatments in those studies indeed affected vocal fold viscosity, which in turn modulated PTP (Verdolini et al., 1994; Verdolini-Marston et al., 1990; see also Verdolini-Marston et al., 1994). However, the arguments are circular because independent estimates of vocal fold viscosity were lacking.

Thus, although the existing data are consistent with the proposed theoretical relations between PTP and vocal fold viscosity, in fact the relations still lack a satisfying empirical test. Some help is provided by recent data showing an increase in the viscosity of excised vocal fold tissue when dry air (0%) was used to induce oscillation (Hemler, Wieneke, Lebacq, & Dejonckere, 2001). However, the applicability to human participants is unclear, as these data ignore in vivo homeostatic regulation, which is a fundamental property of human functioning.

In fact, the principle of homeostasis may be invoked to argue that significant fluid shifts are unlikely in generally healthy humans, who constitute the majority of the population. An implicit assumption is that any shifts that do occur should be minimal and quickly corrected by feedback mechanisms, leaving little if any effective window for vocal or other performance decrements. Another issue is that *if* PTP-induced de/hydration effects are due to fluid shifts in laryngeal tissue, earlier studies failed to elucidate the mechanisms. Systemic or surface (secretory) mechanisms could play a role, or both. From both basic science and clinical perspectives, an understanding of mechanisms underlying de/hydration effects on voice is relevant. Such understanding ultimately should contribute to the development of effective prevention and treatment options for voice problems resulting from dehydration.

Scope of the Study and Experimental Questions

To examine physiological mechanisms underlying dehydration-induced voice changes, the present study serially exposed participants to two different types of dehydration treatments. The goal was to differentially induce systemic versus secretory vocal fold dehydration. Lasix (LA), a potent diuretic, was administered to achieve systemic dehydration, which we estimated from body weight.[1] Diphenhydramine hydrochloride (DH, specifically benadryl), an oral antihistamine, was administered to induce secretory dehydration, which was estimated from saliva viscosity.[2] Sugar pills constituted the placebo treatment.

The central experimental question was: (1) Does systemic or secretory dehydration, or both, mediate PTP increases at high pitches—which have been shown to be particularly sensitive in previous studies (Verdolini et al., 1994; Verdolini-Marston et al., 1990, 1994)? A corollary question was: (2) Do participants detect dehydration-induced PTP changes as changes in perceived phonatory effort?

Methods

Participants

Four healthy college-age adults, two men and two women, were initially enrolled in the experiment. Average age was 25 years (range 21–28 years). All participants denied any remarkable medical history, in particular risk factors of asthma, narrow-angle glaucoma, cardiac disease, renal disease, diabetes, or pregnancy.

[1] The more direct approach of obtaining blood plasma levels to measure systemic hydration was not appealing, because this approach would require repeated or chronic positioning of an arterial line for multiple data collections over a 4-day period that spanned the experimental portion of the protocol. This approach that seemed too invasive for the scope of the experimental question.

[2] Again, the more invasive approach of estimating the viscosity of secretions on the vocal fold surface from direct samples was unacceptable, given the number of samples that would be required (16 samples during each of 3 treatment days).

None took any chronic medications, and all denied any medication use at the time of the experiment. Neither of the female participants menstruated during the protocol, by their report. All participants initially denied any history of a voice disorder, and voice was grossly normal to the examiners at the outset of the experiment. All were naive to the experimental hypotheses; participants were only told that the experiment examined "drug treatment and voice." None had background in clinical voice practice, and all were untrained voice users.

During the protocol, and specifically following one day of Lasix treatment, one day of placebo treatment, and one day off from the protocol, one of the female participants presented for her third and final day of treatment with distinct dysphonia. She indicated that she had first noted subtle hoarseness—which had escaped the examiners—the second day of the protocol (placebo day, which directly followed the Lasix treatment). On the last day of the experiment, when we also noted her hoarseness, a physician investigator examined her larynx. The examination revealed moderate midcordal edema and an hourglass glottal configuration. The participant was immediately discontinued from the experiment and was advised to minimize voice use and to hydrate (Verdolini-Marston et al., 1994). She declined further treatment. Our re-examination 5 days later indicated minimal persistence of edema, with little or no hourglass configuration. Upon further, post hoc questioning, this participant acknowledged that she had indeed experienced morning hoarseness occasionally in the years before the experiment. She had denied any prior voice problem at the outset of the experiment, because she had not considered this a "voice problem."

The occurrence of hoarseness in this participant is interesting because it differs from results for the other participants, who never gave any evidence or history of recurring hoarseness before, during, or after the experiment. The possibility is suggested that even subclinical laryngeal pathology—which the hoarse participant's retrospective history indicated she may have had—may interact in special ways with the combination of dehydration and vocal loading. This is a topic to be pursued in further systematic study.

After the dysphonic participant was discontinued from participation, an additional female participant was enrolled in the protocol. She was 24 years old, and exhibited the same characteristics as other participants, with the exception that she used birth control pills. All participants were compensated monetarily for their participation.

Procedures

Participants first signed approved consent forms and were provided an overview of the protocol. Then they underwent medical examination that confirmed they would be safe to participate. A physician experimenter took a general medical history and inquired about current medications. She examined each patient by assessing body temperature, pulse rate, blood pressure, eyes, ears, nose, neck/throat, heart, lungs, and bicep and Achilles reflexes. After normal medical status was confirmed based on these observations, participants received training in data collection procedures that would be used for PTP and perceived vocal effort measures.[3] At this point and throughout the experiment, measures were elicited by experimenters who were uninformed about the experimental hypotheses and about participants' conditions in the experiment.

PTP procedures were modeled after earlier methodological and empirical reports (Holmberg, Perkell, & Hillman, 1987; Smitheran & Hixon, 1981; Verdolini et al., 1994; Verdolini-Marston et al., 1990, 1994), as follows. With a pressure transducer needle inserted translabially at an angle of approximately 45 degrees, participants were trained to produce the syllable string /pae pae pae pae pae/ at a constant pitch of F4 for men (349.23 Hz), in chest voice, and F5 for women (698.46 Hz), as signaled before each trial by a keyboard and by the experimenter's vocal models. The rate was about 1.53 syllables/second, indicated by a metronome set at 92 beats per minute. High pitches were used because previous research has demonstrated that they are particularly sensitive to de/hydration effects (Verdolini et al., 1994; Verdolini-Marston et al., 1994, 1990). During data collection, participants were instructed to avoid oral occlusion of the transducer needle end and to produce the syllables as quietly as possible, with voicing. Constant reminders were provided to produce voice as quietly as possible. The trials' approximation to actual PTP was indicated by the experimenters' perception that trials were in fact produced as quietly as possible and by the presence of occasional devoiced or whispered syllables. Such trials were excluded from analysis, and were repeated. However, their occurrence was important in confirming a general approximation to PTP. This procedure had been determined in earlier work to produce equivalent phonation trials compared with trials involving sequences of suprathreshold, subthreshold, and threshold attempts (Verdolini-Marston et al., 1990; see also Verdolini et al., 1994). Tokens were accepted as valid when pitch was no more than one-quarter tone deviant from the target pitch, determined perceptually by the trained vocalist data collector, and when the magnitude of visually monitored pressure peaks 2–5 appeared even, with relatively flat tops. Trials not satisfying these criteria or approximating them were eliminated and repeated. On

[3] Participants also received training in the production of automatized Voice Range Profiles, which are not discussed beyond a further footnote in a later section.

this first, no-treatment day, participants repeated 10 repetitions of the 5-syllable strings in each of 4 training sets, described below.

Training for data collection procedures for phonatory effort ratings involved the use of a Direct Magnitude Estimation (DME) scale. This scale was a modification of an earlier one described by Colton and Brown (1972), which indicated a relationship between subglottal pressure and DME ratings and which we have used in other studies on hydration and PTP (Verdolini et al., 1994; Verdolini-Marston et al., 1994). Specifically, after participants completed all PTP trials, they were asked to rate the entire set of trials relative to perceived phonatory effort, on a scale on which 100 represents a comfortable effort. A rating of 200 would represent twice as much effort as comfortable, and 50 would represent only half as much effort as comfortable. Participants were told that there were no upper or lower limits to the scale.

Participants repeated sets of PTP and phonatory effort trials in succession a total of four times, separated by 20-minute intervals, during this initial, no-treatment day. The purpose was to attempt to minimize learning effects in the subsequent data.

Two days later, all participants reported to initiate the treatment phase of the protocol. Treatment orders are indicated in Table 1. In brief, 2 participants received a diphenhydramine hydrochloride (DH) treatment, and 2 participants received a Lasix (LA) treatment during the first treatment day, as described shortly. The following day, participants who had received the LA treatment received a placebo (sugar pill, SP) treatment, and then had one day off before receiving a final day of DH treatment. Participants who initiated the protocol with the DH treatment did not report for the protocol on the second day, but reported the third day for LA treatment and the fourth day for the placebo. The number of intervening days between treatments reflected maximum drug duration times. LA has a peak effect between 60 and 120 minutes, after which most of its effects should be dissipated. Minimal residual effects may be seen as late as 6 to 8 hours postadministration, but these later effects are progressively negligible. Thus, no intervening day was required before the next treatment was administered. In contrast, DH effects peak at about 60 minutes, but reportedly may have minimal progressively dwindling effects as long as 24 hours. To protect from the remote possibility that DH effects might bleed into a subsequent experimental day, one nonexperimental day was inserted after the DH treatment for participants who had not yet completed the protocol.

Across all treatment and placebo days, temporal and measurement aspects of the protocol were identical. The basic protocol is shown in Table 2. Although the protocol was quite straightforward, the time course of events is somewhat challenging to describe. We will attempt to be clear about it here and in the table, because a good understanding of the procedures is key to the interpretation of the results.

In detail, participants reported at 7:00 a.m. (Hour 0 of elapsed time; see Table 2). First, participants completed one full measurement set, as described shortly. The identical set was repeated hourly throughout the day, for a total of 16 measurement sessions each day. In brief, participants first completed PTP trials and phonatory effort estimates, as trained. Because they had previously practiced the PTP task, they rarely failed to meet the task criteria during the experiment proper (<1% of trials throughout the experiment). Thus, only a small number of PTP trials had to be repeated. After PTP and effort trials were completed, participants' blood pressure, pulse, and weight were measured. To limit the variability in weight due to extraneous factors (besides dehydration), participants wore the exact same clothes and ate the same exact foods (in the same quantities ± 10 g) at the same time interval on each day. Participants also maintained the same degree of hair wetness for the first measurement session on each day. Fluid intake will be discussed shortly. After vital signs and weights were measured, participants produced a single 2-cc sputum sample. Some participants required chewing gum to generate the required amount. Gum use was held constant within participants throughout the experiment.[4]

Immediately after the first set of measures had been elicited, at about 7:05 a.m., all participants received two

[4] Also Voice Range Profile (VRP) measures were made. However, the experimental questions were parallel to the ones addressed here. Moreover, the findings were not particularly meaningful. Thus, the VRP will not be further discussed in this paper.

Table 1. Treatment orders. Each sequence was used for 2 participants (1 man and 1 woman). Training occurred 2 days before experimental Day 1. LA is Lasix (diuretic) treatment, and DH is diphenhydramine hydrochloride (antihistamine) treatment.

Number of participants	Training	Day 1	Day 2	Day 3	Day 4
2	Practice	LA	Placebo	***	DH
2	Practice	DH	***	LA	Placebo

Table 2. Elapsed time, clock time, action, and measurement blocks. (See text for explanation of measures.)

Elapsed time in experiment	Time of day	Action	Measurement block
0 hr	7:00 a.m.	Measures	Pretreatment
	7:05 a.m.	Sugar pills	
1 hr	8:00 a.m.	Measures	Pretreatment
2 hr	9:00 a.m.	Measures	Pretreatment
3 hr	10:00 a.m.	Measures	Pretreatment
	10:05 a.m.	Drug or sugar pills	
4 hr	11:00a.m.	Measures	Treatment
5 hr	12:00 p.m.	Measures	Treatment
6 hr	1:00 p.m.	Measures	Treatment
7 hr	2:00 p.m.	Measures	Treatment
	2:05 p.m.	Sugar pills	
8 hr	3:00 p.m.	Measures	Posttreatment
9 hr	4:00 p.m.	Measures	Posttreatment
10 hr	5:00 p.m.	Measures	Posttreatment
11 hr	6:00 p.m.	Measures	Posttreatment
	6:05 p.m.	Sugar pills	
12 hr	7:00 p.m.	Measures	Posttreatment
13 hr	8:00 p.m.	Measures	Posttreatment
14 hr	9:00 p.m.	Measures	Posttreatment
15 hr	10:00 p.m.	Measures	Posttreatment

sugar pills. Measures were then again elicited at 8:00 a.m., 9:00 a.m., and 10:00 a.m. (hours 1, 2, and 3 of *elapsed* time from the start of the experimental day at 7:00 a.m.; Table 2), as previously. Together, the four initial sets of measures (7:00 a.m., 8:00 a.m., 9:00 a.m., and 10:00 a.m.) constituted the "Pretreatment" measures for that day.

About 5 minutes after the last set of pretreatment measures was elicited, i.e., at about 10:05 a.m., the "treatment" for the day was provided. This consisted of either a drug (60 mg of Lasix, distributed in three capsules, on one day; or 50 mg of diphenhydramine hydrochloride, distributed in two capsules, on another day); or two to three sugar pills, depending on a predetermined order for each participant (Table 1). Throughout the experiment, the number of sugar pills in various conditions was systematically varied within and across participants so that participants would not associate pill number with either of the specific drug treatments. To further limit cues about the pills' identities, all pills were housed in identical capsules provided by a local pharmacy. About an hour after a drug or second placebo was administered, at 11:00 a.m., the first set of "Treatment" measures was elicited. The 1-hour time lapse was selected for the first "Treatment" set as peak effects occur during this window for both Lasix (peak effect is at 60–120 min) and diphenhydramine hydrochloride (peak effect is at 60 min). Measures at 11:00 a.m., 12:00 p.m., 1:00 p.m., and 2:00 p.m. constituted "Treatment" measures, because they captured peak effects as well as the major period of dwindling drug effects.[5] Then, for the remainder of the experimental day, until 10:00 p.m., measures again were elicited hourly. After the 2:00 p.m. measures, and again after the 6:00 p.m. measures, each participant received two or three sugar pills, counterbalancing the numbers within and across participants. Measures elicited during this final, 8-hour block (from about 2:05 to 10:00 p.m.) were considered "Posttreatment" measures, as it was anticipated that most if not all appreciable drug effects would be dissipated by that time.

Fluid intake was regulated as follows. During pretreatment sugar pill periods, and during the entire placebo (sugar pill) day, participants were given 8 ounces of fluid (largely water) to drink each hour. This amount was considered a "normal, healthy" quantity for adults to be drinking hourly. On the other hand, when drugs were administered, participants' fluid intake was restricted to 4 ounces of fluid (largely water) per hour for the remainder of the protocol that day. The reason was that we wished to support a situation of relative dehydration in the period immediately following drug administration. We further wished to support a type of drug recovery that we believe is most typical in the clinical population, following inadvertent dehydration.

[5] Declines in drug effects are continuous and gradual. Thus, the decision about what point in time to designate as the start of the "posttreatment" period was somewhat arbitrary, based on information about drug dissipation curves. However, the drug information that we had suggested that the preponderance of drug effects should be expected during the period we designated as the "treatment" period, and those effects should be minimal—if any—during the period we designated as "posttreatment."

Finally, participants were asked to refrain from any food or fluid intake after midnight throughout the protocol and to refrain from alcohol intake at any time during the protocol. Questioning by the experimenters indicated that participants complied with these instructions.

Equipment

PTPs were obtained using the pressure transducer from a Glottal Enterprises MS100-A2 pressure-flow system, with a 15-gauge blunt needle for translabial positioning. The main output of the pressure transducer was routed to a Hewlett Packard 350D attenuator set to 20 dB attenuation for all PTP trials.

Output from the attenuator was routed to a Hewlett Packard 3466A Digital Multimeter and, in parallel, to a Gateway 2000 386 computer. The Multimeter was set on the constant (DC) voltage setting, with a 20-V range. Throughout the experiment, zero input status from the pressure transducer was monitored and the transducer system adjusted, if needed, so that the Multimeter reading indicated less than |0.0005| V. Signals to the computer were imported with an Ariel DSP-32C digital signal processor, with two channels of Motorola sigma-delta analog-to-digital converters. Hypersignal software (1989) was used to capture signals at a rate of 2,000 samples per second.

Pressure signals were calibrated with a Medical Instruments (University of Iowa) U-tube water manometer, marked in cm H_2O and including millimeter markings. Calibration accuracy was ±5 mm H_2O. A Wittner Taktell Piccolo metronome set at 92 beats per minute was used to provide participants with the target rate of syllable production during PTP measure collection. A Casiotone MT-35 portable keyboard was used to provide participants target pitches.

A Westa model scale, precise to ±5 g, was used to weigh participant's food input. For saliva viscosity measures, a model LV7-60228 Wells Brookfield Cone and Plate LV7-60228 model micro-viscometer was used. Saliva vials with individual samples were removed from the refrigerator, and saliva was withdrawn using 1 cc tuberline syringes. The 1 cc of saliva was then deposited into the viscometer cup, which was fastened tightly to the viscometer with a metal clasp. The viscometer's speed control dial was successively set at three speeds: 6 rps, 12 rps, and 30 rps. Three sets of viscosity measures were made for each sample at each speed (6, 12, 30 rps measures were repeated, in that order, three times).

Experimental Design and Data Reduction

The study used a within-subject, double-blind, placebo-controlled crossover design. An important feature of the experimental design was that drug effects (LA, DH) would be detected by comparing time-based fluctuations of the dependent variables (weight, saliva viscosity, PTP, and perceived effort) to fluctuations during the same time contrasts on the placebo treatment day.

Data were entered as follows in the statistical analyses. Weight information (kg) was manually recorded during each measurement session. These data were later entered directly into the analyses.

Saliva viscosity was measured from sputum samples provided during each measurement session. Samples were stored in a refrigerator immediately following collection and were analyzed from 29 to 61 days later. For each sample, 1 cc of sputum was extracted from the 2 cc sample. For each of those samples, in turn, viscosity was measured, in centipoise, three times at each of three different viscometer speeds (6 rps, 12 rps, and 30 rps), using the following formulas:

1. Shear stress (dynes/cm^2) = $T/(2/3)\,\pi r^3$, where T = % scale torque (dyne-cm)
2. Shear rate (s^{-1}) = $\omega/\sin\theta$, where ω = cone speed (rad/s), and
3. Viscosity (centipoise) = shear stress × 100/shear rate

All nine values were used in statistical analyses.

PTP data from each measurement session were analyzed using a software algorithm developed at the University of Iowa. Consistently with prior methodological studies (Holmberg et al., 1987), the algorithm first established the average oral pressures between peaks 2-3, 3-4, and 4-5 of the 5-syllable pressure signal for PTP for each trial. All values were used in later statistical analyses.

Effort ratings that participants provided following PTP trials were manually recorded. The data were later entered in statistical analyses.

Statistical Analyses

For each treatment day (LA, DH, and SP), three time periods or "blocks" were identified (Table 2): (1) a pretreatment block that included four measurement sessions during a 3-hour period of *elapsed* time from the beginning of the experimental day, starting immediately before SP was initially administered; (2) a treatment block that included the 1- to 4-hour period following LA, DH, or SP administration; and (3) a posttreatment block that included the 5- to 12-hour period following LA, DH, or SP administration (Table 2). The justification for the specific time blocks selected was provided in the *Procedures* section. In sum, measurements made between 1 and 4 hours after drug administration would capture most of the drugs' effect. Measurements made between 5 and 12 hours after administration were anticipated to capture residual-to-little effect.

For each of the identified periods, variables relating to possible mechanisms of dehydration (weight and saliva viscosity) and variables relating to vocal function (PTP and effort) were analyzed. As noted, for each measurement session within each block, single estimates of weight and perceived phonatory effort were used in statistical analyses. PTP and saliva viscosity measures involved multiple estimates from each measurement session and thus were handled somewhat differently. For each of the hourly time points per day, 15 PTP measures were used (average pressure across peaks 2-3, 3-4, and 4-5 for each of 5 repetitions of the /pae pae pae pae pae/ string). From those, a mean and standard deviation were obtained to construct the reciprocal variance, to weight that mean and to produce Predicted Population Means for use in the ANOVA for PTP. For viscosity, there were nine measures per hourly time point, from which mean and reciprocal variance weight were obtained. Again, this gave rise to Predicted Population Means that were used in the ANOVA for saliva viscosity.

In an initial analysis, a randomized-block ANOVA with participants as the blocking factor was fit to each of the outcomes (weight, saliva viscosity, PTP, and effort). The experimental factors were treatment type (LA versus DH versus SP), period (pretreatment versus treatment versus posttreatment) and treatment by period interaction. The treatment by period interaction was the variable of interest in this study. The investigation-wide a level across all four interactions (in relation to weight, saliva viscosity, PTP, and effort) was set at $p = .05$. This value underwent a Bonferroni correction, thus setting the a level for each interaction at $p = .0125$ (.05/4). If the interaction was significant at that criterion, nine post hoc comparisons could be performed for the variable in question, including all three pairwise treatment comparisons with all three pairwise period comparisons. With this number of comparisons, the Bonferroni-adjusted alpha was .0056 (.05/9) for each post hoc analysis.

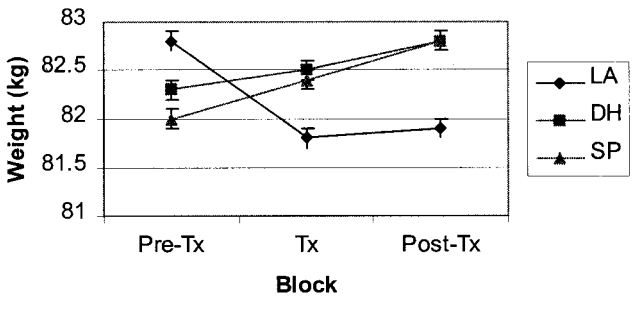
A critical point that we wish to emphasize is that time-based comparisons from the drug conditions (LA and DH) were compared to equivalent time-based comparisons from the placebo condition (SP) and from the alternative treatment (DH or LA). For example, pre-post treatment changes in weight for the LA day were compared with pre-post treatment changes in weight for the SP day, and also with pre-post treatment changes in weight for the DH day. The same logic held for all time-based comparisons, for all dependent variables (weight, saliva viscosity, PTP, and effort). Thus, a significant difference in the time-based comparisons for LA or DH versus SP would imply a drug effect. A significant difference in the time-based comparisons for LA versus DH would imply a differential drug effect.

Results

Magnitudes and Durations of Estimated Systemic and Secretory Dehydration

Predicted Population Means for weight measures reflecting systemic dehydration are displayed in Figure 1 (numeric data are shown in Appendix A). This figure indicates that participants lost approximately 1% of total body weight beginning 1 to 5 hours after the delivery of Lasix (LA) (treatment block) and persisting for the duration of the treatment day (posttreatment block, up to 12 hours after drug administration). Thus, systemic dehydration was accomplished with this treatment. No weight losses were apparent during any aspect of the protocol associated with the antihistamine diphenhydramine hydrochloride (DH) or placebo sugar pill (SP) treatments. In fact, weight tended to *increase* across the day for those conditions, probably due to food and liquid consumed. Statistical analyses indicated that the ANOVA model for the dependent variable of weight accounted for 99.9% of the variance for this parameter. Moreover, the treatment x block interaction was significant [$F(4, 173) = 23.31$; $p = .0001$], indicating that weight changes diverged across treatment days, depending on the treatment given. Post hoc analyses showed that weight changes from LA differed from those from DH or SP. In the LA treatment, weight fluctuations were statistically different from pretreatment to treatment blocks [$F(1, 173) = 37.87$; $p = .0001$] and from pretreatment to posttreatment blocks [$F(1, 173) = 82.78$; $p = .0001$] compared to the placebo control condition (SP). Also, weight fluctuations in the LA condition were significantly different from pretreatment to treatment blocks [$F(1, 173) = 25.67$; $p = .0001$] and from pretreatment to posttreatment blocks [$F(1, 173) = 48.84$; $p = .0001$] compared to the antihistamine condition (DH). In contrast, the DH condition did not show any weight change effects different from SP in any time-based comparison.

Figure 1. Average weight (kg) in pretreatment, treatment, and posttreatment blocks, for Lasix (LA), diphenhydramine hydrochloride (DH), and sugar pill (SP) treatments. Error bars show standard errors.



Predicted Population Means for saliva viscosity, which were assessed as an attempt to capture secretory hydration changes, are shown in Figure 2 (numeric data are in Appendix B). This figure indicates little if any systematic variation in saliva viscosity across any treatment. In fact, statistical analyses failed to reveal any reliable changes in saliva viscosity whatsoever throughout the entire experiment. Neither main effects of treatment or block nor the treatment × block interaction achieved statistical significance. The overall ANOVA accounted for 11.2% of the variance in saliva viscosity.

Phonation Threshold Pressure (PTP) Data

Predicted Population Means for PTP data are displayed in Figure 3 (numeric data are in Appendix C). These data show an increase in PTP in the post-LA treatment block (5 to 12 hours following LA administration) compared with the treatment block, but they show no clear differences in PTP for DH or SP conditions. The overall ANOVA accounted for 86.5% of the variance in average PTP measures. The treatment x block interaction was significant [$F(4, 172) = 3.34$; $p = .0116$], indicating that the diversity across experimental periods diverged across the treatments. Post hoc analyses confirmed that the effects of LA on PTP differed or marginally differed from those of both SP and DH in treatment versus posttreatment comparisons [$F(1, 172) = 7.61$; $p = .0064$; and $F(1, 172) = 8.68$; $p = .0037$ respectively] but that the effects of DH did not differ from those of SP in any block.

Figure 2. Predicted Population Means for saliva viscosity (poise) in pretreatment, treatment, and posttreatment blocks, for Lasix (LA), diphenhydramine hydrochloride (DH), and sugar pill (SP) treatments. Error bars show standard errors.

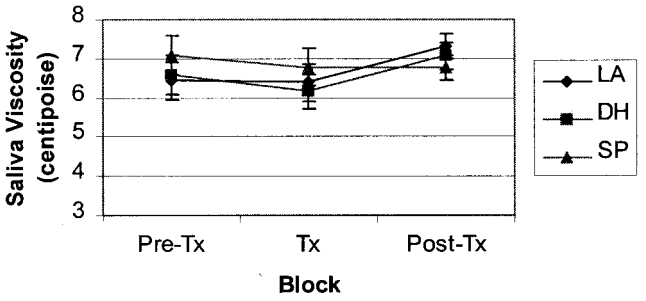
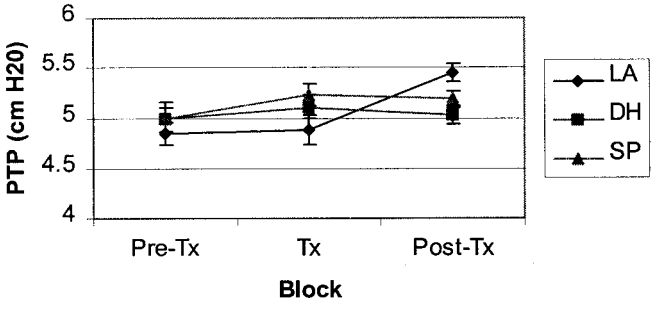


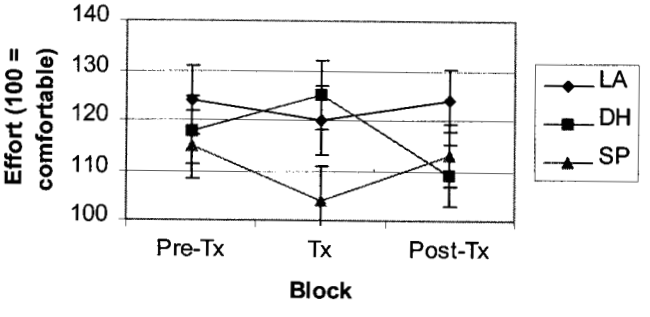
Figure 3. Predicted Population Means for PTP (cm H20) in pretreatment, treatment, and posttreatment blocks, for Lasix (LA), diphenhydramine hydrochloride (DH), and sugar pill (SP) treatments. Error bars show standard errors.



Perceived Effort Data

A secondary question regarded the extent to which participants might perceive increased pulmonary drive (PTP), subjectively, as increased phonatory effort. We start with the presentation of effort data. Average effort data are shown in Figure 4. This figure shows that participants' perception of phonatory effort appeared to decrease somewhat midday during the SP (placebo) day and somewhat less so during the LA (Lasix) treatment day. In contrast, perceived effort did not show the typical midday, treatment-period decrement with the DH (antihistamine) treatment. Rather, in that condition, effort appeared to *increase* midday during the 1- to 4-hour period after DH drug administration. Statistical analyses confirmed an interaction of treatment x block; that is, effort values did appear to vary across the day in the different conditions [$F(2, 178) = 3.73$; $p = .0061$]. Post hoc comparisons indicated that the only reliable findings involved greater perceived effort immediately following the DH treatment in relation to the posttreatment period compared to the same time-based changes in placebo (SP) and Lasix (LA) treatments [$F(1, 178) = 13.67$; $p = .0003$ when comparing treatment versus posttreatment blocks for DH versus SP conditions; $F(1, 178) = 7.87$; $p = .0056$ when comparing treatment versus posttreatment blocks across DH and LA]. The interpretation is not entirely clear as a result of a lack of reliable

Figure 4. Average effort measures (DME scale; 100 = "comfortable effort") in pretreatment, treatment, and posttreatment blocks, for Lasix (LA), diphenhydramine hydrochloride (DH), and sugar pill (SP) treatments. Error bars show standard errors.



differences between pretreatment- and treatment-period blocks for any of the compared conditions (LA vs. SP; LA vs. DH; or DH vs. SP); thus, a statistical paradox is presented. The overall picture that emerges is that phonatory effort was minimal midday during the placebo (SP) condition, perhaps due to warm-up and practice effects. Fatigue or other factors then caused an increase in effort towards morning baselines in the evening. Lasix (LA) apparently did little or nothing to change this pattern. In contrast, the antihistamine (DH) appeared to counteract normal midday decreases in effort, causing it to increase compared to apparent morning baseline. As the drug window passed, effort then returned to roughly baseline values, which were similar to those seen in the SP and LA conditions.

More to the point for our particular experimental question, little if any covariance was seen between perceived phonatory effort and physiological effort measures (PTP). A comparison of Figures 3 and 4 makes this point. PTPs reliably increased, in relation to placebo, only from treatment to posttreatment periods following Lasix administration (Figure 3). Effort did not increase correspondingly during this time-based comparison. Moreover, effort measures appeared to be relatively large during the treatment period for the antihistamine (DH). PTP measures were not increased in that condition.

Discussion

Magnitude and Duration of Systemic and Secretory Fluid Perturbation and Their Relation to PTP

A one-time, single-dose systemic dehydration treatment, Lasix (LA), produced weight losses on the order of about 1% of total body mass. Thus, systemic dehydration was achieved by the experimental manipulations. Systemic dehydration effects were noted during the 1- to 4-hour block after the drug was administered, as anticipated by pharmaceutical specifications (Lambright Eckler & Stimmel Fair, 1996). An unanticipated finding was that weight losses persisted in our study for as long as participants were observed during the LA treatment day (5–12 hours after drug administration). Weight losses following LA were significantly different from weight fluctuations in a placebo condition.

Reliable PTP shifts on the order of about 23% relative to baseline also occurred following LA—but not following DH or SP administration. Fatigue is not a likely explanation, as fatigue should have occurred uniformly across all conditions, which required identical vocal tasks across the same time periods on all days. It is acknowledged that an interaction of LA and fatigue could have contributed to the results. However, overall, the combined findings imply a role of systemic vocal fold water content in the regulation of PTP. The most interesting aspect of those results is the delay between whole-body dehydration and PTP changes. PTP shifts were first clearly apparent during a 5- to 12-hour block after drug administration. Thus, they lagged behind weight losses by several hours.

Before reviewing the results from our attempts to dehydrate participants' secretions, we first proceed with a discussion of the systemic effects. An overview of systemic hydration mechanisms in general may be useful. Ingested fluids are absorbed by intestinal cells, which transfer water and other material to the extraluminal capillary network. The kidneys filter the fluid by balancing water and salts, primarily Na^+ and Cl^-. The vascular highway then delivers water to extra- and intracellular tissues throughout the body. Osmotic gradients determine all fluid flow across endothelial, extracellular, and intracellular spaces. With relevance to the extracellular matrix (ECM), which comprises the critical oscillatory material in the vocal folds, the normal structural transport vehicle is capillary leakage.

In our study, weight loss and the associated phonatory effects were induced by furosemide (Lasix), which is a high-ceiling, loop diuretic. This class of drugs, which inhibit $Na^+ –K^+ –2CL^-$ symport in the kidney's thick ascending limb of the loop of Henle, is particularly potent in peak effect. Furosemide increases not only water but also Na^+ and usually Cl^- excretion. By inhibiting Na^+ and Cl^- resorbtion in the nephron, these ions are excreted, causing an acute reduction in total circulatory volume and thus extracellular fluid loss. In sum, it seems likely that much if not most of the drug-induced weight losses in the present study could be attributed to inhibition of NaCl transport to the ECM (see, for example, Jackson, 1996).

The point is that a single dose of a relatively common diuretic was effective to induce a persistent diuresis in our healthy participants. An important observation is that although humans are homeostatically regulated, homeostasis implies an inertial feedback mechanism with a built-in time delay. Fluid correction does not occur simultaneously to fluid losses, but rather—as implied by our data—may require hours or longer. Our data indicate that within that interval, there is ample time for measurable performance disruptions. In fact, vocal disruptions in the form of PTP shifts did occur in our study within the systemic dehydration window.

A second important point from our data is that, assuming that PTP changes were caused by local fluid changes in the vocal folds' ECM, vocal fold changes were asynchronous with whole-body changes. This observation illustrates another important property of homeostasis: although healthy humans maintain constant

whole-body hydration averaged over extended time frames (e.g., 24 hours; see for example, Beck, 1971), fluids readily and constantly traverse endothelial, extracellular, and intracellular environments within that time frame (Beck, 1971). Thus, although long-term average whole-body dehydration predicts long-term average focal hydration, whole-body hydration cannot predict fluid contents in any specific body part *at a given point in time*. The results from our study suggest that the respiratory system including the vocal tract, may retain fluids longer than some other body parts when systemic losses do occur. However, even the respiratory system ultimately is affected in a way that disrupts phonation if dehydration is not corrected.

A final comment before turning to a discussion of these results' potential applicability regards the dependence of PTP on fluid shifts. The results are *consistent* with the proposal that an increase in vocal fold extracellular matrix viscosity modulated PTP. However, alternative explanations also are possible. Diuretic drugs can produce deficits in neuromuscular function above and beyond those due to fluid loss (viscosity) (McArdle, Katch, & Katch, 1996). Furthermore, fluid loss itself has the potential to alter neuromuscular performance in addition to altering tissue viscosity. For example, fluid losses of about 1% of total body mass, in the range shown by our participants, increase the exercise heart rate by about 8 beats per minute and decrease cardiac output by about 1.0 L/min (McArdle et al., 1996). Decreased neuromuscular endurance is one result (McArdle et al., 1996). A thorough discussion of the effects of dehydration-induced performance decrements is beyond the scope of this paper. At this juncture, the point is that alternative explanations besides viscosity changes are possible to explain PTP shifts with systemic dehydration. Direct in vivo measures of tissue viscosity versus neuromuscular function may help to clarify these issues experimentally.

Regarding the results' applicability, at least two questions are relevant. First, to what extent do findings for diuretics apply to voice users in general, or persons with voice disorders? We think that there are parallels. First of all, diuretics are commonly encountered in the population at large, including persons with voice problems. Diuretics are used to treat edema from an array of medical conditions such as hypertension, congestive heart failure, nephrotic syndrome, liver cirrhosis, chronic renal insufficiency, corticosteroid therapy, open-angle glaucoma, and postoperative brain surgery, in addition to epilepsy, mountain sickness, drug overdose, and hypercalcemia (Jackson, 1996). Data from our author group indicate that similar or even heightened PTP changes compared to those we found here occur in hypervolemic patients undergoing clinically indicated fluid removal (Fisher, Ligon, Sobecks, & Roxe, 2001). Additionally, some individuals use diuretics for weight control. Equally relevant, systemic dehydration is a distinct risk factor for large sectors of the population not undergoing specific diuretic therapy. Examples include persons with transient diarrhea, elderly age (e.g., Dardaine, Ferry, & Constans, 1999), infant age (e.g., Beck, 1971), endogenous or pharmaceutical sympathetic nervous system arousal from use of adrenergic sympathomimetic or anticholinergic drugs, and in athletes (e.g., Coyle, 1999). Specific to this last category, in generally healthy, exercising persons who may be encountered in voice caseloads, fluid losses from sweating during intense exercise in hot environments can be as great as 3 L/hour and can average about 12 L (26 lb) daily. The point is that frank homeostatic fluctuations in fluid balance and dehydration not only occur, but arguably occur relatively commonly in the population at large, including persons with voice disorders. Our data demonstrate that the correction of fluid imbalance is anything but instantaneous even in healthy participants, leaving ample windows for voice performance decrements.

Regardless of the specific mechanisms, our data indicate that vocal effects of systemic dehydration can be seen with a lag—in our data of several hours—in the form of PTP increases. In persons subjected to repeated or chronic systemic dehydration, effect magnitudes and durations might be expected to be even greater than those shown here for one-time single-dose administration of diuretics to healthy participants.

One final word about the clinical applicability of the results regards our use of high pitches to detect dehydration-induced PTP effects at high pitches. We used high pitches because these are known to be most sensitive to de/hydration effects, which we wanted to detect (Verdolini et al., 1994; Verdolini-Marston et al., 1990; see also Verdolini-Marston et al., 1994). Clearly, high pitches per se are of limited relevance to most of the population. However, virtually all speakers modulate pitch during speech, and pitches nearly as high as those used here (F4 for men, F5 for women) may occur during normal speech. Further, our clinical impression is that high pitches can sometimes capture relevant vocalization parameters that patients note at other pitches but that are otherwise poorly appreciable (see for example, Bastian, Keidar, & Verdolini-Marston, 1990).

In contrast to the findings for systemic dehydration, we failed to achieve any evidence of secretory drying following antihistamine (DH) administration, based on estimates from saliva viscosity. This finding is somewhat curious, as DH is an antihistamine that is an H1 receptor antagonist with strong anticholinergic properties (Rau, 1998). As such, salivary and bronchial secretions that bathe the vocal folds should be reduced with this drug. Perhaps saliva viscosity is unaffected by respiratory

drying. Or perhaps the dosage of antihistamine that we delivered (one-time dosage of 50 mg of DH) was insufficient—or the wrong time-frame was assessed—to capture appreciable drying effects in the saliva. Another possibility is that although we did not find any evidence in the literature of a time window beyond which saliva viscosity changes its potency when stored refrigerated, arguably some loss of viscosity potency did occur in our study due to the relatively long storage times between sample collections and data extraction (29–61 days). Whatever the reasons, the bottom line is that not only did measured saliva viscosity fail to change with any of the experimental conditions in our study, also PTP showed no changes from the treatment that we expected might alter saliva viscosity.

Of course, the obvious point is that significance cannot be imputed to null results. We can only observe that we did not achieve evidence of secretory dehydration or PTP effects with the antihistamine, not that the effects cannot be obtained. Other studies have reported increases in PTP as well as deteriorated voice output with a vocal fold surface drying induced with dessicated air (Hemler, Wieneke, & Dejonckere, 1997; Jiang et al., 2000).

Covariance of Respiratory Effort (PTP) to Perceived Effort in Phonation

A secondary experimental question was whether participants might perceive dehydration-induced changes in physiological effort as increased psychological effort. At one level, the answer appeared to be "no." Participants did not perceive increases in pulmonary effort (PTP) that did occur as increases in psychological effort. This finding is somewhat perplexing in light of previously reported covariances of the variables, specifically following dehydration (Verdolini et al., 1994). However, even in the earlier work, the correspondence was incomplete at best. In that study, perceived phonatory effort correctly detected PTP increases in dehydation treatments; however, effort failed to distinguish moist versus control conditions, which PTPs clearly had differentiated (Verdolini et al., 1994). In another study of participants with nodules, perceived effort robustly distinguished hydration from placebo treatments in the anticipated direction (Verdolini-Marston et al., 1994). However, PTPs were insensitive to condition in that study, and thus a correspondence between the measures was not shown.

Recent work by Colton provides some clarification about relations between perceived phonatory effort and PTP. He found that the relation between perceived effort and lung pressure in excess of threshold was r = .671. This value was somewhat stronger than the relation considering threshold pressure alone (R. H. Colton, personal communication). Thus, it is clear that although subglottic pressure and PTP may contribute to phonatory effort, other causal contributors also exist.

One candidate for another possible contributor to perceived phonatory effort was, in fact, suggested by our data: central nervous system effects. Our data showed weak evidence of an increase in perceived phonatory effort during the 4-hour block following antihistamine (DH) administration. Because this perception was not paralleled by PTP increases, assuming the effect is real, other factors must have caused it. Diphenhydramine hydrochloride (DH) has a series of documented side effects, including drowsiness, lethargy, fatigue, muscle weakness, dizziness, drop in blood pressure, ataxia, and headache (Lambright Eckler & Stimmel Fair, 1996). It cannot be ruled out that such effects could account for the increase in perceived phonatory effort following DH administration. However, the finding was somewhat weak and the explanation is speculative. Clearly, further research is needed on this topic.

Summary

This study provided evidence consistent with the possibility that systemic dehydration from diuretics may increase vocal fold tissue viscosity internally, mediating PTP increases at high pitches—which are particularly sensitive to hydration changes—with a lag of several hours. Systemic drying associated with these changes most likely occurs in the extracellular matrix, although alternative possibilities are not excluded.

In contrast to the findings for systemic dehydration, no evidence was obtained of secretory dehydration with a single dose of an antihistamine. Similarly, no evidence was obtained of an increase in PTP at high pitches with that drug. Possibly, effects might be obtained with greater dosages, different measurement windows, and/or longer-duration drug administration than we used. Other evidence does suggest that laryngeal tissue viscosity can increase, and voice deteriorations can occur, when dry air is delivered to the larynx of both excised samples and human participants (Hemler et al., 1997, 2001; Jiang et al., 2000). Moreover, our participants may have perceived phonatory effort increases following antihistamine administration. The mechanisms are unclear, and the issue is somewhat tangential to the primary purposes of this study. However, as a note of interest, central nervous system effects cannot be ruled out as an explanation for this incidental finding.

Finally, participants did not reliably detect increases in dehydration-induced pulmonary effort (PTP) as increased phonatory effort. Thus, based on our data, it seems that clinical reports of phonatory effort are not necessarily reliable indicators of hydration status.

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Appendix A. Weight (kg).

		Treatment					
		Placebo (SP)		Lasix (LA)		Benadryl (DH)	
Block		Mean	Standard error	Mean	Standard error	Mean	Standard error
Pretreatment	Participant 1	80.14		81.38		80.37	
	Participant 2	99.90		100.75		100.25	
	Participant 3	69.33		70.17		69.47	
	Participant 4	78.57		78.78		79.08	
Group Mean		82.0	0.1	82.8	0.1	82.3	0.1
Treatment	Participant 1	80.88		80.38		80.67	
	Participant 2	100.03		100.10		100.68	
	Participant 3	69.78		69.33		69.88	
	Participant 4	78.78		77.55		78.85	
Group Mean		82.4	0.1	81.8	0.1	82.5	0.1
Posttreatment	Participant 1	80.93		80.19		80.44	
	Participant 2	100.49		100.34		101.15	
	Participant 3	70.44		69.23		70.15	
	Participant 4	79.31		77.75		79.29	
Group Mean		82.8	0.1	81.9	0.1	82.8	0.1

Appendix B. Predicted Population Means for Saliva Viscosity (poise).

		Treatment					
		Placebo (SP)		Lasix (LA)		Benadryl (DH)	
Block		Mean	Standard error	Mean	Standard error	Mean	Standard error
Pretreatment	Participant 1	9.21		6.43		8.18	
	Participant 2	6.52		6.33		6.32	
	Participant 3	6.07		6.58		6.79	
	Participant 4	6.38		7.28		6.90	
Group Mean		7.08	0.50	6.43	0.49	6.57	0.50
Treatment	Participant 1	9.33		6.14		7.81	
	Participant 2	5.84		7.10		5.77	
	Participant 3	6.61		6.78		7.18	
	Participant 4	6.79		8.45		5.74	
Group Mean		6.77	0.49	6.38	0.47	6.15	0.43
Posttreatment	Participant 1	10.31		7.25		7.78	
	Participant 2	6.27		7.31		7.56	
	Participant 3	6.32		6.82		6.76	
	Participant 4	6.48		8.80		6.91	
Group Mean		6.75	0.32	7.31	0.34	7.07	0.34

Appendix C. Predicted Population Means for PTP (cm H_2O).

		Treatment					
		Placebo (SP)		Lasix (LA)		Benadryl (DH)	
Block		Mean	Standard error	Mean	Standard error	Mean	Standard error
Pretreatment	Participant 1	3.52		3.44		3.18	
	Participant 2	4.35		3.97		4.29	
	Participant 3	7.90		8.30		6.73	
	Participant 4	4.92		5.05		5.62	
Group Mean		4.99	0.12	4.85	0.11	5.00	0.16
Treatment	Participant 1	3.59		3.06		3.82	
	Participant 2	4.95		4.21		4.49	
	Participant 3	6.91		6.87		6.44	
	Participant 4	4.82		5.82		5.25	
Group Mean		5.23	0.11	4.88	0.14	5.11	0.12
Posttreatment	Participant 1	3.54		3.94		3.66	
	Participant 2	4.85		5.00		4.55	
	Participant 3	6.98		7.91		6.34	
	Participant 4	5.27		5.28		4.96	
Group Mean		5.19	0.07	5.45	0.10	5.02	0.08

Appendix D. Effort Ratings (100 = comfortable effort).

		Treatment					
		Placebo (SP)		Lasix (LA)		Benadryl (DH)	
Block		Mean	Standard error	Mean	Standard error	Mean	Standard error
Pretreatment	Participant 1	108.75		122.50		110.00	
	Participant 2	122.50		122.50		130.00	
	Participant 3	125.00		137.50		125.00	
	Participant 4	102.50		115.00		107.50	
Group Mean		115	6.75	124	6.75	118	6.75
Treatment	Participant 1	90.0	0.0	105.75		113.33	
	Participant 2	120.00	0.0	120.00		123.33	
	Participant 3	106.25	6.25	137.50		131.25	
	Participant 4	100.00	0.0	117.50		127.50	
Group Mean		104	6.75	120	6.75	125	6.90
Posttreatment	Participant 1	98.75	2.27	102.50		115.00	
	Participant 2	130.00	5.75	132.50		118.13	
	Participant 3	118.75	4.09	153.13		112.50	
	Participant 4	106.25	3.24	106.25		91.25	
Group Mean		113	6.20	124	6.20	109	6.20