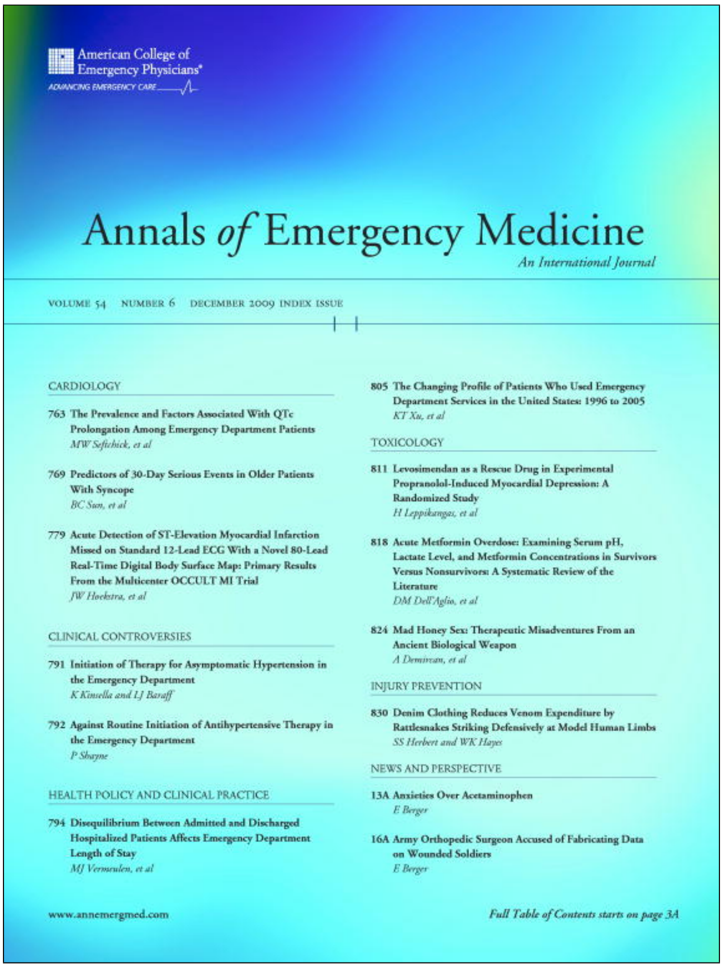
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Full Table of Contents starts on page 3A

Denim Clothing Reduces Venom Expenditure by Rattlesnakes Striking Defensively at Model Human Limbs

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Study objective: Venomous snakebites can be painful, costly, and potentially life threatening. We seek to learn whether ordinary clothing (denim material from blue jeans) interferes with the kinematics of venom delivery, thereby reducing the amount of venom injected by a representative viper into a human limb.

Methods: In a laboratory study, we used model human limbs (warm, saline solution–filled gloves) to elicit defensive strikes from small and large southern Pacific rattlesnakes (*Crotalus oreganus helleri*). Each snake was videotaped biting a bare glove and a denim-covered glove.

Results: The snakes injected significantly less venom into denim-covered gloves than bare gloves during defensive strikes, with a 60% reduction for small snakes and 66% for large snakes. Latency to bite, number of bites, and duration of fang contact during the bite were similar for the 2 glove types, suggesting that the 2 targets elicited similar defensive behaviors and strikes. Several findings suggested that denim interfered with venom delivery, including the high proportion of dry bites for denim-covered gloves and the large quantity of venom spilled harmlessly on the denim cover. Large rattlesnakes struck more readily, maintained longer fang contact during the bite, and delivered 26 to 41 times more venom into gloves than small snakes.

Conclusion: In our model, denim clothing proved effective at reducing venom injection by both small and large rattlesnakes. Wearing long denim pants as an alternative to shorts may provide a simple, low-cost means of reducing the severity of snakebites. [Ann Emerg Med. 2009;54:830-836.]

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INTRODUCTION

As human populations continue to expand and encroach on the habitats of venomous snakes, encounters potentially resulting in human envenomations will persist.[1-3] Recent studies suggest that 400,000 to 2 million snake envenomations occur globally each year, resulting in 20,000 to 100,000 deaths and countless more cases of long-term disability.[1,4-7] The consequent personal and financial costs of snakebite to individuals, families, and communities can be staggering.

Any practical solutions that might reduce the frequency or severity of snakebites and associated costs warrant evaluation. Preventative measures should begin with alertness to one's surroundings and awareness of habitats and conditions that favor snake encounters.[2] First aid measures should be understood, with proficiency in applying the appropriate measures and avoiding those that are inappropriate.[8-10] Footwear or clothing also can be worn that protects against fang penetration[11,12] or reduces the amount of venom injected.[13] Any reduction in venom delivery would reduce the severity of envenomation.[14,15]

Numerous products are sold that purportedly protect against snakebite, including penetration-resistant pants, chaps, gaiters, and boots. However, these products are worn primarily by snake specialists,[16] and their use can impede efficient movement through snake habitats. Although ordinary clothing (eg, long pants, long-sleeved shirts) is vulnerable to snake fang penetration,[11] it may also provide a measure of protection, particularly when long pants rather than shorts are worn in snake habitat.

The purpose of this study was to test whether ordinary clothing typically worn by a human significantly reduces the amount of venom injected by a rattlesnake during a defensive bite of a model human limb. In doing so, we also considered how the potential protective effect might vary with snake size.

Editor's Capsule Summary

What is already known on this topic

There are few proven methods to reduce the severity of venomous snakebite.

What question this study addressed

The authors used an in vitro model to determine whether less venom was injected when a snake struck a latex glove covered with denim than when the snake struck a latex glove lacking denim.

What this study adds to our knowledge

The denim-covered gloves received roughly one third the venom received by the "naked" glove.

How this might change clinical practice

Because definitive proof in humans will likely never be obtained, this commonsense approach can be endorsed for routine use in humans.

We chose to test whether denim material—the material frequently used for long pants, or "blue jeans"—might reduce the severity of envenomation resulting from a bite to a model of a human leg. A denim barrier may interfere with the kinematics of a bite by deflecting the fangs, disrupting jaw and fang movements, altering fang penetration depth and trajectories, and mistiming venom expulsion.[14,17,18] We further hypothesized that the disruptive effect of clothing would be greater for juvenile snakes with shorter fangs compared with adult snakes.

MATERIALS AND METHODS

The viperid snakes used in this experiment were eight small (35- to 54-cm snout to vent length) and nine large (66- to 102-cm snout to vent length) southern Pacific rattlesnakes (*Crotalus oreganus helleri*). Snakes were individually maintained in assorted cage sizes with a light:dark cycle of 14:10 hours at 25 to 27°C (77 to 81°F). Each cage included pine shavings for substrate and a glass vessel containing water ad libitum. The snakes were fed laboratory mice (*Mus musculus*) every 2 weeks (13 to 15 days) and were fasted at this interval before each strike trial. Animal use in this study was approved by the institutional animal care and use committee.

We prepared 2 conditions to elicit defensive bites. The first was a bare human limb model composed of a prerinsed household latex glove (30-cm length, 0.6-mm thickness; catalog no. 534557; Napa, Inc., Napa, CA) filled with 500 mL of warm (38°C) phosphate-buffered saline solution (pH 7.4) and secured with a plastic zip tie. The latex provided minimal leakage of fluid after fang penetration and withdrawal. The glove was also rubbed against the investigator's arms to transfer human scent. The second was identical, except that the glove was loosely covered with a single layer of clean (prerinsed in deionized water and dried) denim material. In both conditions, the model was suspended from an aluminum snake hook by an additional zip tie for presentation to the snake. The model was able to swing freely from the hook.

Snakes were individually transferred by snake hook to a 1×1×0.6-m (length×width×height) wooden arena with a fresh 1×1-m craft paper floor covering and allowed 5 minutes for acclimation. The arena was lighted from above by three 100-W bulbs within metal reflectors approximately 1.25 m above the floor. Each snake was tested twice, once with the bare glove and once with the denim-covered glove, with 2 weeks separating the trials. The sequence of presentation was randomized such that half the snakes were assigned the bare glove first and half were assigned the denim-covered glove first. Trials were recorded by an S-VHS camcorder (Panasonic PV-S7700-A; Kadoma, Osaka, Japan) at standard tape speed (30 fields/second), with a 1/500-second shutter speed. The camera was positioned at approximately 1.25 m obliquely above the arena.

Presentation of the glove was standardized to consist of approximately 5 seconds of noncontact harassment, followed by a thrust of the model toward the snake (but avoiding contact). This sequence was repeated until a bite occurred or until 15 minutes elapsed, at which point the trial was terminated. On some occasions, the snake managed to bite the glove twice before we could retrieve it. In all trials, the snake behaviors and strikes elicited were unambiguously defensive, accompanied by considerable rattling, head-elevated body coiling, and prolonged arcing tongue flicks interrupted by occasional escape crawling.[19] Immediately after a bite, the glove was transferred to a clean 1-L beaker, whereupon the denim cover was removed if present and placed within a plastic zip-lock bag. The phosphate-buffered saline solution–filled glove was gently mixed (rocked back and forth) to ensure even distribution of the venom and then dumped into the beaker for further mixing before a 10-mL sample was transferred by plastic transfer pipette into a plastic test tube. Occasional fluid spillage through fang punctures in the gloves was deemed a trivial source of venom loss. The denim covers were then placed in 400-mL phosphate-buffered saline solution and agitated for 2 minutes before another 10-mL sample was transferred into a plastic test tube. Both the glove and denim cover samples were frozen at –20°C for subsequent venom assays.

A total protein assay (Coomassie 1 to 25 μg/mL protocol; Pierce Chemical Co., Rockford, IL) was used to quantify venom in the experimental samples. Accomplishing this required appropriate control samples to derive standard curves. Control standards for the glove samples were created by injecting 7 prewashed bare gloves containing 500 mL phosphate-buffered saline solution with different quantities of *C atrox* venom (0, 20, 40, 60, 80, and 100 mg dissolved in 0.5 mL phosphate-buffered saline solution at pH 7.4; Kentucky Reptile Zoo, Slade, KY) using a tuberculin syringe and 22-gauge needle. These control gloves and samples derived from them were treated in a manner identical to the experimental gloves, including handling of gloves with bare hands. Control standards for the denim covers were created from 6 clean (prerinsed in deionized water and allowed to dry) denim covers. The denim covers were placed in a beaker and then loosely injected by syringe and needle with 0, 2, 4, 8, 10, and 12 mg *C atrox* venom dissolved in 0.5 mL phosphate-buffered saline solution. The denim covers were subsequently treated in a manner identical to the experimental gloves, except that they were not placed into and manipulated within the strike arena.

Experimental and control glove samples were assayed together, in triplicate, on 96-well microtiter plates (catalog no. 430247; Corning Inc., Corning, NY). Experimental and control denim cover samples were likewise assayed together, in triplicate, on plates separate from the glove samples. Absorbance values (570 nm) from the controls were used to generate separate standard curves for the glove and denim cover samples. The standard curves were used to estimate the dry mass of venom (mg) injected by snakes, using linear regression equations. When absorbance values from experimental samples exceeded those from the standard curve, experimental samples were diluted up to 10-fold and assayed again. Calculations of venom mass from diluted samples were adjusted to reflect their original concentration. The coefficients of determination for the standard curves were indicative of the high reliability of the assays (all $r^2 \geq 0.88$).

During frame-by-frame videotape review, we recorded for each strike trial the latency to bite, number of bites, and duration of fang contact with the model (defensive strikes by rattlesnakes almost always involve a quick bite and release). In some cases, incomplete video records (camcorder not turned on when glove was introduced to the arena, or the glove obscured the snake's biting actions from the camera) reduced the sample size for behavioral variables. From the protein assays, we determined the mass of venom expended (nearest milligram, dry mass) in each glove model and denim cover. Because controls indicated that denim covers picked up a consistent average of 8 mg extraneous protein from the strike arena, we subtracted this amount when determining their venom content. For models covered with denim, we computed the proportion of venom spilled on the denim (venom spilled on the denim divided by sum of venom injected into glove and venom spilled on the denim). Because our primary interest was whether denim reduced the amount of venom injected into the target, we did not adjust the mass of venom expended for the few targets that received multiple bites.[20]

Primary Data Analysis

We used SPSS 13.0 for Windows (SPSS Inc., Chicago, IL) to conduct analyses. To meet parametric assumptions, we rank-transformed the duration of fang contact, all measures of venom expended, and the proportion of venom spilled on the denim. We conducted 2×2 mixed ANOVAs,[21] for which glove condition (bare, denim covered) was treated as a within-subjects factor and snake size (small, large) as a between-subjects factor. For ANOVAs, effect sizes were obtained as partial η^2 values,[22] indicating the approximate proportion of variance in a dependent variable explained by each independent variable or interaction, with values greater than 0.25 generally considered large. When partial η^2 values for main effects and interactions summed to greater than 1.0, we adjusted these by dividing each partial η^2 by the sum of all partial η^2 values. For tests of proportions, we relied on nonparametric χ^2 and McNemar tests, followed by Φ for effect size,[23] with a value greater than 0.5 deemed large. We used a 2-tailed paired *t* test,[24] with effect size computed as Cohen's *d* with pooled SD,[22] for which values greater than 0.8 are generally considered large. We also used Pearson correlation, expressed as the coefficient of determination (r^2) to indicate effect size, with a value greater than 0.25 deemed large.[22] α Levels of 0.05 were used for all tests.

RESULTS

A total of 31 bites were obtained from the 17 snakes. However, sample sizes for most statistical tests were limited to the 5 small and 7 large snakes that had complete venom data for both glove conditions, as required for related data. Three snakes were excluded because one of the glove conditions was mishandled (ie, fluid spilled), and 2 snakes refused to strike at both conditions; partial data obtained from these 5 snakes were consistent with those from snakes having complete data. Comparisons between the 2 size classes and 2 glove conditions for most dependent variables can be viewed in the Table.

An ANOVA revealed no differences in duration of harassment before biting between the 2 glove conditions ($F_{1,8}=0.07$; $P=.80$; partial $\eta^2=0.01$) and between the 2 snake size classes ($F_{1,8}=4.53$; $P=.066$; partial $\eta^2=0.36$). However, the effect size for snake size class was substantial, suggesting that large snakes struck more quickly than small snakes (means=1.3 and 3.7 minutes, respectively, when pooled for both glove conditions; Table). There was no interaction between glove condition and snake size ($F_{1,8}=0.12$; $P=.74$; partial $\eta^2=0.06$).

The majority of trials involved single defensive bites because we tried to avoid eliciting multiple bites. However, 2 rapid bites occurred in one (6.7%) of the 15 trials involving bites by small snakes and 3 (18.8%) of the 16 trials involving bites by large snakes. After pooling of bites by small and large snakes within each glove condition, a McNemar's test for snakes having complete data revealed no difference in the proportion of trials involving multiple bites between bare (8.3% of 12 trials) and denim-covered (25% of 12 trials) gloves (exact 2-tailed $P=.63$; $\Phi=0.17$), though the moderate effect size suggests that denim-covered gloves received more bites.

Mean values of fang contact were highly skewed by 3 strike trials involving difficulty with fang disengagement, resulting in durations much longer than the typical 0.20 to 0.25 seconds for strikes at gloves by large rattlesnakes.[14] One trial for a bare glove included 2 bites that required 5.33 seconds total, and 2 trials for denim-covered gloves involved single bites requiring 2.63 and

Table. Mean (SD) values for variables associated with defensive bites by small and large southern Pacific rattlesnakes (*C. oreganus helleri*).

Dependent Measures	Bare Glove		Denim Glove		Denim Cover		Denim Total (Glove+Cover)	
	Small Snakes	Large Snakes	Small Snakes	Large Snakes	Small Snakes	Large Snakes	Small Snakes	Large Snakes
Latency to bite, min	3.6 (2.8) (N=5)	1.7 (2.0) (N=5)	3.7 (3.0) (N=5)	1.0 (1.3) (N=5)	—	—	—	—
Fang contact duration, s	0.12 (0.06) (median=0.10) (N=4)	1.19 (2.31) (median=0.18) (N=5)	0.19 (0.06) (median=0.18) (N=4)	2.11 (3.08) (median=0.33) (N=5)	—	—	—	—
Venom expended, mg	4 (3.4) (range 0–8) (N=5)	164 (112.6) (range 15–358) (N=7)	2 (2.1) (range 0–5) (N=5)	56 (66.8) (range 0–146) (N=7)	—	14 (19.7) (range 1–55) (N=7)	—	70 (81.9) (range 1–188) (N=7)

The targets were warm, saline solution-filled gloves (model human limbs) that were either bare or covered by denim clothing; venom spilled on the denim covers could not be reliably measured for small snakes.

7.29 seconds for disengagement. All other strike trials involved fang contact of less than or equal to 0.33 seconds, including 3 more involving double bites. Accordingly, rank-transformed data were used for statistical analysis and median values are reported with the means in the Table. The ANOVA yielded no differences between the 2 glove conditions ($F_{1,7}$=1.74; P=.23; partial η^2=0.20), though the moderate effect size suggests that fang contact was longer for denim-covered gloves. The significant difference for size class ($F_{1,7}$=6.89; P=.03; partial η^2=0.50) indicated that large snakes maintained longer fang contact than the small snakes (median=0.20 and 0.13 seconds, respectively, when pooled for both glove conditions; Table). There was no interaction between glove condition and size class ($F_{1,7}$=0.02; P=.90; partial η^2 <0.01).

For venom injected into gloves (Table), which should correspond to venom injected into human tissues, the significant effect of glove condition in an ANOVA confirmed that snakes delivered approximately two thirds less venom into the denim-covered gloves than into the bare gloves (small snakes before rounding to nearest 1 mg, 60% less; large snakes, 66% less; $F_{1,10}$=6.47; P=.029; adjusted partial η^2=0.35). Snake size was also significant, with large snakes injecting 41 and 26 times more venom into bare and denim-covered gloves, respectively, than the small snakes ($F_{1,10}$=14.86; P=.003; adjusted partial η^2=0.54). These effect sizes were large despite considerable variation in quantities of venom injected (Table). There was no interaction between these variables ($F_{1,10}$=1.39; P=.27; adjusted partial η^2=0.11), suggesting that glove interference with venom injection was similar for the 2 size classes.

For bites of denim-covered gloves, we determined the amounts of venom spilled harmlessly on the denim and total venom expended (glove+cover) only for large snakes (Table). Amounts recorded for small snakes (range=8-10 mg) could not be reliably distinguished from extraneous proteins picked up from the test arena (8 mg). For total venom expended, a paired *t* test showed that the large snakes expended near-equal amounts of venom for the 2 glove conditions (t=1.87; df=6; P=.11; Cohen's d=0.82), although the large effect size suggests that more venom was injected into the bare gloves. These snakes also spilled an average of 14 mg of venom on the denim, representing 43% (1 SD=39%; range=5% to 100%) of the snake's total venom expenditure. The proportion of venom spilled on the denim was negatively associated with the total amount of venom expended (large snakes: r^2=0.41, N=7, P=.12). Although this correlation was not significant because of the small sample size, the effect size was large.

From the 29 bites from which we were able to measure venom, there were obvious differences in the proportion of dry bites (<0.5 mg venom injected into glove) between glove conditions and between size classes of snakes. When snakes of all sizes were considered together, bare gloves (7.7% of 13 bites) received proportionally fewer dry bites than denim-covered gloves (31.3% of 16 bites); however, because analysis of related data was restricted to snakes with complete data (omitting some of the dry bites), the difference between glove conditions was not significant (McNemar's test, exact 2-tailed $P=.63$, $\Phi=0.17$). When trials were pooled for the 2 glove types, small snakes (38.5% of 13 bites) delivered proportionally more dry bites than large snakes (6.3% of 16 bites; $\chi^2=4.54$, $df=1$, asymptotic $P=.033$, $\Phi=0.40$). Among the small snakes, a notable proportion of bites to denim-covered gloves (50% of 8 bites) was dry compared with strikes at bare gloves (20% of 5 bites), suggesting that denim was particularly likely to interfere with venom delivery for small snakes.

LIMITATIONS

Several limitations apply to this study. We designed the experiment primarily to test whether ordinary clothing reduces venom injection during a bite and not precisely by how much. The amount of venom a snake injects when biting defensively varies with many factors other than clothing, with snake size being most important.[14,18] Considerable variation in venom expenditure by snakes during defensive bites must be anticipated, and substantial envenomation through clothing can still occur,[11] sometimes resulting in death. Some fabrics and clothing designs undoubtedly provide more protection than others,[25] and careful evaluation of these might prove profitable. We tested only one fabric type. The models differed from human limbs in several respects that could influence venom injection, including their more pliable nature (fluid content), reduced resistance to venom flow from the fang tips,[18] and kinetic properties (swinging from a snake hook). These model attributes were nevertheless consistent between the 2 conditions. The experiment was also designed to avoiding pinning or restraining the snake. Snakes of some species grasped by a human and provoked to bite inject more venom under this high level of threat than when they are unrestrained,[14,18,26] but the only available data for rattlesnakes suggest that they do not.[27,28] Provocation or handling of a snake influences the anatomic site of the bite, with many such bites being delivered to upper limbs.[29,30] Although we studied a single representative viper species, we anticipate that ordinary clothing could reduce venom injection for most, if not all, venomous snake species. The protective effect might even be greater for the comparatively short-fanged elapid and venomous colubrid snakes, which warrants further study. Finally, the small sample sizes in this study render some conclusions tenuous, particularly those supported by smaller effect sizes.

DISCUSSION

Our results suggest that, from the perspective of a potential snakebite victim, wearing ordinary long pants as an alternative to shorts when in snake habitat can substantially reduce the amount of venom a snake injects during a defensive bite. The reduction in venom injected into denim-covered model human limbs was approximately two thirds for both small (60%) and large (66%) rattlesnakes. As a consequence, the average severity of envenomation for human snakebite victims could be reduced by wearing clothing that covers the limbs. A less severe bite, in turn, would reduce the costs associated with treatment and the risks of complications leading to surgery and long-term morbidity.

What caused the reduction of venom injected by snakes into the model human limbs? Two possibilities could be considered: either the snakes perceived and responded to the 2 targets differently or the denim covering interfered with venom delivery.

Regarding the first possibility, which invokes venom metering (decisionmaking) by the snakes,[18] several findings in our study suggest that the snakes responded similarly to the 2 targets. The 2 targets presented different visual-thermal images to the snakes, which might have affected the snakes' perception of and defensive responses to the threat. However, the 2 conditions elicited statistically similar behaviors from the snakes in terms of latency to strike, number of bites delivered, and duration of fang contact. Thus, the differences in venom injection should not have resulted from target features overtly affecting prestrike behaviors, the tendency to launch strikes, or bite duration, the latter being a key kinematic variable that affects venom delivery during defensive bites.[14,18,27] A subsequent unpublished study confirmed that rattlesnakes respond similarly to cool and warm gloves and inject comparable amounts of venom into them (W. K. Hayes and Z. Nisani, unpublished data, 2007).

Regarding the second possibility, other results support our interpretation that clothing interfered with venom delivery by the snakes. First, for the denim-covered gloves, a high proportion of venom was spilled harmlessly onto the denim. This amounted to 43% of the total venom expended by large snakes. Some venom undoubtedly was lost on the surface of bare gloves, which we did not measure, but we saw very little spilled venom (the venom is bright yellow). Second, more venom was delivered into the bare gloves during a period of fang contact equal to or less than that for the denim-covered gloves. The denim covering likely reduced the proportion of time that fangs were in contact with the glove itself, thereby reducing the amount of time that venom could be injected into the glove.[14,18] Third, a negative correlation existed between total venom expended for denim-covered gloves and the proportion of venom spilled onto the denim. Thus, when fangs cleanly penetrated the denim, resulting in more efficient venom delivery, the snakes appeared able to eject a larger bolus of venom. Some of the venom measured on the denim may have resulted from inadvertent venom loss as the snake struggled to disengage its fangs. Although not significant statistically, the moderate effect sizes suggest that snakes delivered more bites and bites of longer fang contact to the denim-covered gloves, perhaps in response to the kinematic difficulties associated with venom delivery. In spite of these opportunities to introduce additional venom, the snakes still injected substantially less venom into the denim-covered gloves.

Differences in the frequency of dry bites further suggest that denim interfered with venom injection. The proportion of dry bites to denim-covered gloves (31.3%) was greater than that to bare gloves (7.7%), though analysis of snakes with complete data did not support this statistically. The denim appeared to be particularly problematic for smaller snakes, resulting in an exceptional proportion of dry bites (50%). However, there may have been bias in detecting dry bites of smaller snakes because the amounts of venom normally expended are much closer to the threshold for a dry bite (<0.5 mg venom) than for adult snakes. Although dry defensive bites may result from venom metering by the snake,[14,18] many of the dry bites in the present study presumably resulted from kinematic or target penetration constraints, particularly those delivered to the denim-covered gloves.

Despite popular belief in the United States,[14] a growing body of evidence clearly indicates that large venomous snakes, including rattlesnakes, are much more dangerous to humans than small ones. In some venomous species, larger snakes whose bites serve a more effective antipredator deterrent will strike more readily than smaller snakes,[3] as supported by the large effect size for time to strike in the present study; however, this may not be characteristic of all taxa or defensive contexts.[31] Larger snakes often strike with greater velocity, distance, and accuracy.[3,31,32] Larger snakes also maintain longer fang contact with the target during the bite,[32] as supported by the difference observed in the present study (but see Herbert[27]). Larger snakes inject substantially more venom than smaller snakes,[14,18,27,33] as supported by the present findings, and larger snakes inflict more serious envenomation in humans.[14,15,29] Greater venom expenditure by larger snakes results from having more venom available[34] and greater rates of venom flow through larger-diameter ducts and fangs.[14,27] Thus, the more effective antipredator deterrent of bites from larger snakes may explain why they resort to biting more readily than smaller snakes.

In conclusion, in our model, denim clothing proved effective at reducing venom injection by both small and large rattlesnakes. Wearing long denim pants as an alternative to shorts may provide a simple, low-cost means of reducing the severity of snakebites.

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Author contributions: WKH conceived the study and provided statistical advice. SSH and WKH designed the experiment, analyzed the data, and prepared the article. SSH executed the experiment. WKH takes responsibility for the paper as a whole.

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