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ARTICLE

# Clinical evaluation of large volume subcutaneous injection tissue effects, pain, and acceptability in healthy adults

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## Abstract

Determining feasibility and tolerability of large volume viscous subcutaneous injection may enable optimized, intuitive delivery system design. A translational early feasibility clinical study examined large volume subcutaneous injection viability, tolerability, acceptability, tissue effects and depot location for ~1, 8, and 20 cP injections at volumes up to 10 ml in the abdomen and 5 ml in the thigh in 32 healthy adult subjects. A commercial syringe pump system delivered 192 randomized, constant rate (20 μl/s) injections (6/subject) with in-line injection pressure captured versus time. Deposition location was qualified via ultrasound. Tissue effects and pain tolerability were monitored through 2 hours post-injection with corresponding Likert acceptability questionnaires administered through 72 hours. All injection conditions were feasible and well-tolerated with ≥79.3% favorable subject responses for injection site appearance and sensation immediately post-injection, increasing to ≥96.8% at 24 hours. Mean subject pain measured via 100 mm visual analog scale increased at needle insertion (6.9 mm, SD 10.8), peaked during injection (26.9 mm, SD 21.7) and diminished within 10 minutes post-removal (1.9 mm, SD 4.2). Immediate injection site wheal (90.9%) and erythema (92.6%) formation was observed with progressive although incomplete resolution through 2 hours (44.6% and 11.4% remaining, respectively). Wheal resolution occurred more rapidly at lower viscosities. Most subjects (64.5%) had no preference between abdomen and thigh. Correlations between tissue effects, injection pressure and pain were weak (Pearson's rho ± 0–0.4). The large volume injections tested, 1–20 cP viscosities up to 10 ml in the abdomen and 5 ml in the thigh, are feasible with good subject acceptability and rapid resolution of tissue effects and pain.

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Traditional chronic disease intravenous therapy is transitioning to subcutaneous administration, creating viscous formulations delivered at volumes exceeding the

traditional threshold of 1.5–3.0 ml. Understanding the local physiological impact and corresponding tolerability of large volume subcutaneous injection is key to optimized, intuitive delivery system design.

**WHAT QUESTION DID THIS STUDY ADDRESS?**

Early feasibility clinical to determine feasibility and tolerability of constant rate (20 μl/s), 1–20 cP, 5–10 ml subcutaneous placebo injections to the thigh and abdomen in healthy adults. Are injections and corresponding local tissue effects visible, acceptable, and quick to resolve?

**WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?**

Localized tissue effects (wheals and erythema) and pain are common but transient with broad, favorable acceptability for subcutaneous injections up to 10 ml and 20 cP. Pain peaks during injection, returning to pre-injection levels within 30 minutes. Tissue effects are larger and slower to resolve for thigh and/ or higher volume and viscosity injections.

**HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?**

The tolerability and feasibility boundaries of large volume subcutaneous administration include and likely exceed the injection conditions tested.

## INTRODUCTION

Biological therapies for chronic disease are transitioning from traditional, in-clinic intravenous administration to subcutaneous administration to reduce cost, time, and adverse systemic effects while providing increased patient autonomy and convenience.[1–10] Conditions currently treated with large molecule and biological therapies include but are not limited to cancer, skin disorders, and autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, and primary immunodefic iency.[1,5,11–15]

Adapting intravenous formulations for subcutaneous administration presents various challenges, such as larger than traditional 1.5–3 ml subcutaneous injection volumes,[16–21] solubilizing biotherapeutics at high concentration with shelf-life stability, and higher formulation concentrations, viscosities, and/or altered dosing to accommodate lower subcutaneous versus intravenous bioavailability.[1,13,15,16,22–24] Biologicals currently requiring high-volume subcutaneous administration include but are not limited to trastuzumab for HER2-positive breast cancer, rituximab for non-Hodgkin's lymphoma and various immunoglobulins for primary immunodeficiency.[1,5,8,13–15]

While multiple devices are in development to accommodate large volume subcutaneous (LVSC) injection formulations, limited options are commercially available.[16,25–27] Some formulations indicated for LVSC delivery may utilize multiple injections or permeation enhancers.[17] Recombinant human hyaluronidase is a known permeation enhancer that transiently cleaves hyaluronic acid in the subcutaneous extracellular matrix to improve tissue permeability and absorption.[1,5,8,10,16,28,29] Current use of hyaluronidase requires pre- or co-administration, potentially increasing delivery process complexity and/or formulation requirements, which may not be optimal for all delivery situations.

Understanding of LVSC injection biomechanical requirements, physiological feasibility, and subject tolerability are required to develop effective and safe delivery devices.[27] Using translational methodology to emulate various delivery parameters, confirm LVSC injection feasibility, and provide tolerability data can inform critical mechanical design and engineering factors for integrated LVSC injection device development.[27] Surrogate delivery systems, such as the programmable infusion pump in the current study, enable examination of a wider range of LVSC injection variables, including volumes, viscosities, formulations, and rates or pressures than may be readily feasible with a fully integrated single use delivery device.[26,27] Prior studies in literature have effectively used pump-driven injections over duration times ranging from 10 seconds to 20 minutes to explore single bolus placebo injections up to 20 ml and viscosities up to 20 cP.[1,5,17,20,26,30] However, published studies around the breadth of potential LVSC injection conditions and their clinical response remain limited.[16]

The goal of the current early feasibility clinical study was to examine LVSC injection across a range of injection volumes, sites, and viscosities for achievability, subject tolerability, corresponding tissue impact, and in-line injection pressures. Syringe pump-driven, 20 μl/s constant rate 5 and 10 ml injections ($n = 192$) at 1.1, 8, and 20 cP viscosities were delivered to the abdomen and thigh (5 ml only)

of 32 human subjects split equally between sexes. Site tissue effects (wheal, erythema, bleeding, and bruising) and subject tolerability via visual analog scale (VAS) pain scores were monitored through 2 hours post-injection; corresponding subject acceptability was documented via questionnaires (Likert responses) through 72 hours post-injection. Injectate deposition location was qualified via ultrasound. In-line injection pressure during delivery was also analyzed.

## MATERIALS AND METHODS

### Study design

This early feasibility clinical study was a single-center (Eurofins Optimed, Gières, France), open-label partial crossover study designed to evaluate the feasibility, acceptability, and tolerability of LVSC injections at volumes up to 10 ml and viscosities up to 20 cP delivered in the abdomen and thigh of 32 healthy adult subjects balanced between sexes. Study inclusion and exclusion criteria dictated that subjects were healthy, 18–65 years of age with body mass index (BMI) ≥18.5 kg/m$^2$, and without pregnancy, chronic illness, sensitivity to adhesive or placebo, visible injection site skin condition/ disease, scars, or tattoos, or having taken anticoagulants or analgesics within 12 hours of injections.

Trial subjects were seen twice in-clinic: visit 1 for informed consent, screening, and enrollment and visit 2 for all injections and assessments. Subjects in recumbency each received six injections, one per injection condition in Table 1. Injections were administered one at a time, a minimum of 1 hour apart in a randomized sequence over four abdominal quadrants around the umbilicus and in each of the right and left anterior thigh. All device placements, injections, and assessments were performed by trained health care providers (HCPs). Not all possible combinations of viscosity, volume, and injection site were examined in the study design to ensure adequate physical distance between injection site locations and sufficient time between injections during a single clinical visit to avoid confounding subject perception responses. Excessive hair was trimmed at the injection site if necessary.

The protocol was approved by the Ethics Committee (CPP Sud Est IV, Lyon, France) and French health agency, Agence nationale de sécurité du médicament, under trial registration number 2016-A00326-45. Study was conducted in accordance with the Declaration of Helsinki (October 2013) and Good Clinical Practice guidelines.

**TABLE 1** Injection number per injection condition and delivered volume data

| Injection condition | Site | Volume (ml) | Injection solution | Viscosity* (cP) | *N* total injections | *N* delivered ±20% | Mean delivered volume** ml (SD) | Mean percent delivered volume** % (SD) |
|---|---|---|---|---|---|---|---|---|
| A | Abdomen | 5 | Saline | 1.1 | 32 | 28 | 5.2 (0.2) | 104.1 (4.7) |
| B | Abdomen | 5 | 10% HA | ~17, 8 | 32 | 29 | 5.2 (0.1) | 103.8 (2.9) |
| C | Abdomen | 5 | 20% HA | ~88, 20 | 32 | 29 | 4.9 (0.2) | 98.4 (4.2) |
| D | Abdomen | 10 | 20% HA | ~88, 20 | 32 | 29 | 10.2 (0.3) | 102 (2.5) |
| E | Thigh | 5 | Saline | 1.1 | 32 | 31 | 5.4 (0.13) | 107.3 (2.6) |
| F | Thigh | 5 | 20% HA | ~88, 20 | 32 | 29 | 4.9 (0.2) | 98.4 (4.5) |

**For injections delivering ±20% of the target volume. *Viscosity measured at 20°C at 2 shear rates simulating "in-vial" at 20–25 s$^{-1}$ and "under flow" at ~1000 s$^{-1}$. HA is a non-Newtonian fluid.
Abbreviation: HA, hyaluronic acid.

## Sample size determination

A two sample paired *t*-test (assumed correlation of 0.6, power 80%, alpha = 0.05) based on historic clinical human pain VAS scores determined a minimum of 28 subjects would be needed in order to detect clinically significant differences of 10 mm.[30,33–38] A total of 32 subjects were enrolled to ensure the minimum number of completed injections per injection condition. Subjects were compensated for their participation.

## Delivery system and solutions

All components of the delivery system used herein were commercially available and Conformité Européenne (CE)-marked. No investigational devices or products were used in this study. The surrogate delivery system used to evaluate various delivery conditions consisted of a Harvard PhD Ultra 4400 CP syringe pump (Harvard Apparatus), 60 ml syringe (BD) and a Contact Detach 32" infusion set with 6 mm, 29GA stainless steel cannula (Unomedical). The infusion set was manually inserted perpendicular to skin by trained HCP's per manufacturer instructions and remained in situ post-injection through a 10 minute pressure equilibration period prior to device removal. The syringe pump was set for constant rate delivery (20 μl/s) for both 5 and 10 ml injections (4.1 and 8.2 minute delivery durations, respectively). Thigh delivery was limited to 5 ml volumes only. An in-line blood pressure transducer (DTX TNF-R; Argon Medical Devices) was placed between the syringe and infusion set and connected to a data acquisition system (National Instruments) controlled by a laptop to measure fluid path injection pressures. Fluid line pressure was collected at 1 Hz frequency from injection start through the 10 minute pressure equilibration. The equilibration interval was previously optimized internally to ensure complete 5 or 10 ml ±20% volume delivery across all injection conditions. Device removal post-10-minute equilibration was designated as 0 hours post-injection.

Injection solutions were a 1.1 cP 0.9% weight per volume (w/v) physiological saline and a non-crosslinked commercial hyaluronic acid (HA; Vivacy Laboratories) diluted to 10% and 20% volume in the physiologic saline to reach nominal viscosities of 8 and 20 cP (~1000 $s^{-1}$, 20°C). The HA is CE-marked and nonanimal in origin. HA solutions were prepared daily and measured for density and viscosity at two shear rates (~25 $s^{-1}$ and ~1000 $s^{-1}$) at 20°C. The HA solutions exhibit non-Newtonian shear thinning behavior at increasing shear rate; viscosity (Table 1) is reported at both shear rates to reflect in-vial and during-flow viscosity estimates.

## Tissue effects assessments

All injection sites were confirmed to have no visible tissue effects, tattoos, or scars pre-injection. Tissue effects were evaluated at 0, 0.5, 1, and 2 hours post-injection. If wheal formation was observed, HCP's measured wheal length (major axis) and width (minor axis) with calipers.[17,18,31] Wheal dimensions were used to calculate wheal area based on theoretical optimum elliptical geometry. For purposes of this study, observed wheals were likely due to tissue distension from fluid deposition, rather than other causes.

Erythema was assigned a grade of none, very slight, well-defined, moderate or severe to characterize the visible observations; the grading scale was adapted from prior guidelines.[10,32] Erythema scores reflect all potential composite causes related to injection condition and device wear.

A similar five-point observational grading scale qualified observed bleeding as none, tinge of red, drop of red, oozing blood, or significant bleeding. The frequency of bruising was also noted (yes/no).

## Subject tolerability, acceptability, and preference

Subject pain (tolerability) was quantified using a standard 100 mm VAS (0 mm no pain to 100 mm worst pain)[33] prior to needle insertion (naïve), immediately after needle insertion, during injection (Table 2), at pump stop, and at 0 (device removal after 10 minute equilibration), 0.5, 1, and 2 hours post-injection. For purposes of this study, the minimum clinically significant difference (MCSD) between VAS scores was defined as 10 mm.[30,33–38]

Subjects completed acceptability and preference questionnaires (Table 3) at 0, 0.5, 1, and 2 hours post-injection during visit 2 in the clinic and via follow-up telephone interviews at 24, 48, and 72 hours. Acceptability responses were answered with a five-point Likert scale: strongly agree, agree, neutral, disagree, and strongly disagree.[39]

## Deposition imaging

Ultrasound imaging at each site was performed with a DERMCUP portable ultrasound with linear probe (Atys Medical) pre-injection for baseline tissue morphology and post-injection for depot location by an experienced ultrasound clinician. Depot location in tissue was qualitatively classified from visual ultrasound examination as intradermal, subcutaneous, intramuscular, or some combination thereof.

**TABLE 2** VAS pain score (0–100 mm) summary data collected during injection (peak)

| VAS pain scores (mm) during injection | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Injection condition** | **Volume (ml)** | **Site** | **Solution** | **Viscosity* (cP)** | ***N*** | **Mean (mm)** | **SD (mm)** | **Median (mm)** |
| A | 5 | Abdomen | Saline | 1.1 | 28 | 34.3 | 20.2 | 36.7 |
| B | 5 | Abdomen | 10% HA | 8 | 29 | 28.6 | 22.4 | 24.9 |
| C | 5 | Abdomen | 20% HA | 20 | 29 | 19.6 | 16.0 | 19.1 |
| D | 10 | Abdomen | 20% HA | 20 | 29 | 34.6 | 25.2 | 30 |
| E | 5 | Thigh | Saline | 1.1 | 31 | 24.8 | 23.1 | 20.8 |
| F | 5 | Thigh | 20% HA | 20 | 29 | 19.7 | 18.0 | 14.4 |

Abbreviations: HA, hyaluronic acid; VAS, visual analog scale.

**TABLE 3** Acceptability questionnaire agreeable (favorable, agree, + strongly agree Likert) responses at 0 hour post-injection

| Questionnaire results: Total % agreeable (favorable, agree + strongly agree Likert) responses at 0 hour | | | | | | |
|---|---|---|---|---|---|---|
| | **Abdomen** | | | | **Thigh** | |
| **Question** | **5 ml 1.1 cP** | **5 ml 8 cP** | **5 ml 20 cP** | **10 ml 20 cP** | **5 ml 1.1 cP** | **5 ml 20 cP** |
| I feel no pain | 92.9 | 93.1 | 89.7 | 86.2 | 96.8 | 96.6 |
| I feel no itching | 100 | 96.6 | 100 | 100 | 96.8 | 96.6 |
| I feel no burning | 100 | 93.1 | 96.6 | 89.7 | 96.8 | 100 |
| I feel no pressure | 96.4 | 89.7 | 89.7 | 79.3 | 83.9 | 93.1 |
| I feel no soreness | 96.4 | 93.1 | 93.1 | 93.1 | 100 | 100 |
| The appearance is acceptable | 92.9 | 96.6 | 93.1 | 82.8 | 96.8 | 93.1 |
| Location preference | 3.2 | | | | 32.3 | |

*Note:* Responses were per five-point Likert scale (strongly disagree, disagree, neutral, agree, and strongly agree). The final line shows injection site preference (no preference/ abdomen/ thigh) answered after all six injections received; 64.5% of subjects had no site preference between thigh and abdomen.

## Delivered volume and fluid loss

Delivered volume was assessed by gravimetric analysis of the complete fluid path pre- and post-injection, including fluid loss collected during line priming or post-infusion set removal. Injection leakage observable on the skin surface post-injection was also collected and weighed.[40] Delivered volume was confirmed using the formula: (pre-injection weight – post-injection weight – all fluid collection)/solution density.

## Safety assessments

All injections were considered for safety and adverse event (AE) reporting. Normally expected LVSC injection effects documented as study endpoints through visit two, such as transient local pain during injection or tissue effects, were not documented as AEs but as study endpoints detailed in the results. However, such effects were reported as AEs if the principal investigator judged their severity or nature to exceed that normally associated with the LVSC injection procedure or if the events persisted or manifested beyond the observation interval during visit two.

## Statistical methods

Only injections that delivered ±20% of the target 5 or 10 ml delivered volume were included in the statistical analysis to ensure injection volume equivalency. Statistical analysis software was R version 3.3.1 (R Foundation for Statistical Computing). Comparisons of injection conditions for the various endpoints of interest were performed using nonparametric Wilcoxon pairwise multiple comparison with Bonferroni's adjustment applied when appropriate. This was done after observing non-normality of the residuals for mixed effect models with injection condition, time post-injection, and their interactions as fixed effects and subject as random effect. Injection order was also evaluated as a fixed effect but was not found a significant contributing factor. Correlations were either Pearson's (continuous) or Spearman's (categorical) rho.

# RESULTS

## Demographics

Fifty-one subjects were screened. Thirty-two healthy adult subjects were enrolled and completed the study, each receiving six injections (4 abdomen and 2 thigh), one per injection condition in Table 1. Subjects were evenly distributed between sexes with a mean age of 31.7 years (SD 9.8, range 18 to 52 years) and mean BMI of 23.3 kg/m$^2$ (SD 3.2, range 19.5 to 29.3 kg/m$^2$). Subjects were predominantly Caucasian/White ($n = 30$). See Table S2 for subject disposition and demographic summary.

## Delivered volume and fluid loss

A total of 192 injections were administered with 175 (91%) meeting the ±20% 5 or 10 ml target volume (Table 1). The included injections ($n = 175$) delivered a mean of 102.38% (SD 4.85%) of the 5 and 10 ml target volumes with a minimal mean fluid loss of 0.93% (SD 1.22%) during and post-injection. There were no occlusions.

Seventeen injections were excluded due to incomplete delivered volume ($n = 12$ <80% target volume delivered), equipment issues ($n = 2$) or unconfirmed delivered volumes ($n = 3$).

## Tissue effects

Wheal formation was observed at 90.9% of injection sites immediately post-injection with a mean area of 15.03 cm$^2$ (SD 9.3; Table S1). The wheal area reflects the total visible footprint of the wheal. Resolution was more rapid at lower viscosities but remained incomplete across all injection conditions after 2 hours at 44.6% of injection sites with measurable wheals (mean area 10.66 cm$^2$, SD 10.64; Figure 1, Table S1). Wheals were not observed at >50% of 1.1 cP sites after 1 hour or 8 cP sites after 2 hours. Highest viscosity (20 cP) wheal resolution was 48.3% for 5 ml abdomen, 31% for 10 ml abdomen, and 27.6% for 5 ml thigh injections after 2 hours. Wheal data trends suggest that larger viscosities, volumes, and thigh injections may have longer resolution times (Figure 1, Table S1). The 20 cP abdominal 10 ml and thigh 5 ml wheals were not significantly different from each other but were most consistently larger than the 5 ml abdominal 1–20 cP injection conditions through 2 hours ($p$ value ≤ 0.021). The 1.1 cP 5 ml thigh injection best illustrates the impact of site location; while its measurable wheal frequency (Figure 1 left) is similar to its abdominal counterpart, its mean wheal area (Figure 1 right) is more comparable to the 10 ml 20 cP abdominal and 5 ml 20 cP thigh wheals at 0 hour post-injection, remains larger than the 5 ml 1–20 cP abdominal wheals through 1 hour and is not significantly different than any injection condition through 2 hours despite its lower viscosity and volume.

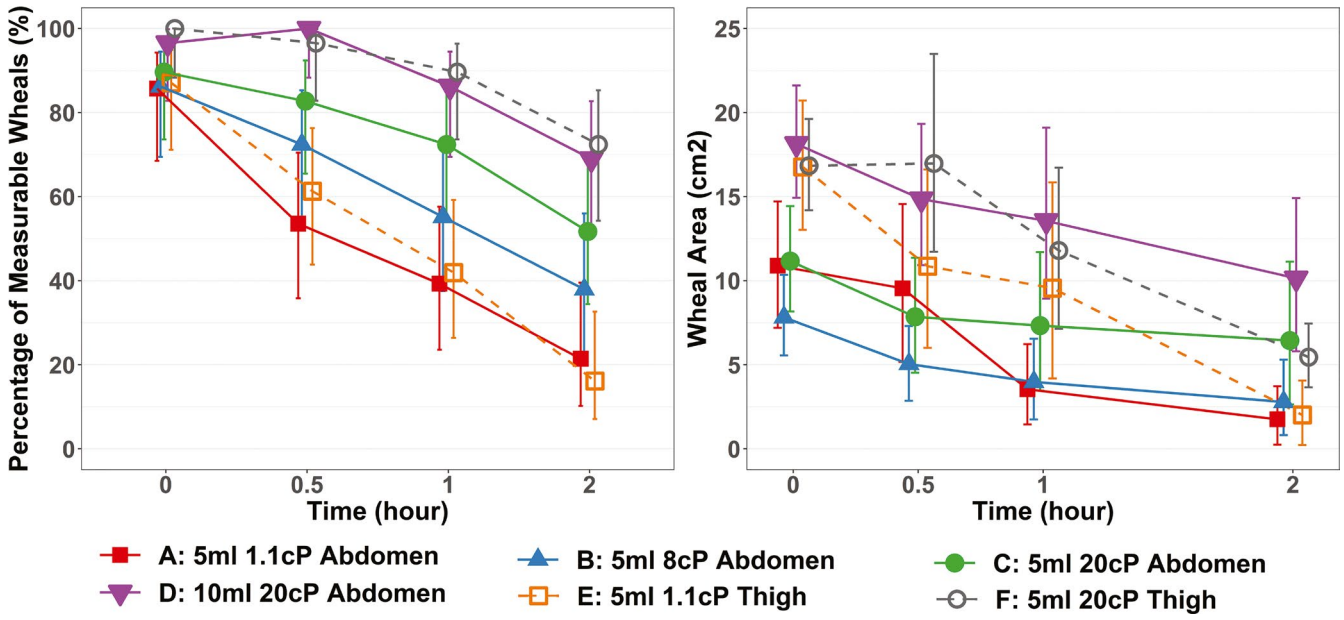


**FIGURE 1** Frequency (%) of measurable wheals with 95% confidence interval (CI; calculated using Wilson's score method, left) and corresponding wheal area (cm$^2$, right) mean and 95% CI (calculated using bootstrap) per injection condition and time post-injection. The wheal area represents the entire footprint (spread) of wheal formation and is calculated from the measured wheal length (major axis) and width (minor axis) dimensions using theoretical elliptical geometry. Connecting lines between data points are solid for abdominal injections (A 5 ml 1.1 cP, B 5 ml 8 cP, C 5 ml 20 cP, and D 10 ml 20 cP) and dashed for thigh injections (E 5 ml 1.1 cP, F 5 ml 20 cP)

Very slight (58.3%) to well-defined (34.3%) erythema was observed at 92.6% of all sites post-injection (0 hour) with complete resolution for 62.3% of sites at 1 hour and 88.6% at 2 hours. Erythema intensity was significantly greater for abdominal 10 ml 20 cP injection sites than the 5 ml thigh injections (0 hour only, $p$ value ≤ 0.047); no significant differences existed between other injection conditions or at subsequent time points. No erythema more intense than well-defined was observed.

Minor bleeding of grades 1–2 (tinge to a drop of blood) was observed at 36.6% of injection sites. Bleeding resolved immediately for all but 4% or fewer sites that had an observed tinge of red at subsequent assessments. Bruising was observed at three sites total, one site per each of the 0, 0.5, 1, and 2 hour timepoints, with one site unresolved at 2 hours post-injection.

Five subjects reported some erythema, a nodule or ecchymosis/hematoma at 1–2 of their injection sites during the 24–72 hour follow-up telephone interviews. The subject-reported tissue effects were considered nonserious with no additional in-clinic assessment deemed necessary by the principal investigator. No subjects were seen in-clinic after visit two.

A single nonserious, mild intensity AE was recorded in one subject with transient urticaria 45 minutes after device removal for a 1.1 cP 5 ml abdominal injection; resolution was complete within 1 hour 15 min and did not require additional follow-up.

## Injection pain (tolerability) and acceptability

Mean VAS scores rose from naïve baseline (0.02 mm, SD 0.19), after needle insertion (6.9 mm, SD 10.8), to peak during injection (26.9 mm, SD 21.7) and at pump stop (24.8 mm, SD 21.9). Pain diminished rapidly, falling below needle insertion levels at 0 hour (1.9 mm, SD 4.2, pump stop +10 min) and returning to naïve baseline at 30 minutes post-injection (0.4 mm, SD 1.6). There were no clinically significant differences (≥10 mm) between the injection conditions, and correlation to tissue effects and injection pressure was weak (Pearson's rho ±0–0.04).

Although differences were neither clinically statistically significant nor the data normally distributed, the trend for median and mean peak VAS scores during injection for 5 ml abdominal injections suggest that pain decreased as the viscosity increased and the concentration of saline decreased (Figure 2, Table 2): 1.1 cP (saline) > 8 cP (10% HA, 90% saline) > 20 cP (20% HA, 80% saline). Trends also suggest that abdominal injections may be more painful than thigh for 5 ml, 1.1 cP (saline) injections but are more equivalent for 5 ml, 20 cP (20% HA, 80% saline) injections (Table 2). Additionally, abdominal 20 cP injection data trends during injection and at pump stop (end of injection) suggest 10 ml volumes may be more painful than 5 ml.

Subject responses were 79.3%–100% agreeable (agree plus strongly agree Likert responses) regarding the absence of injection site pain, itching, burning, pressure, or soreness and the acceptability of their injection site appearance at 0 hours post-injection (Table 3). Agreeable responses increased to ≥96.3% by 24–72 hours post-injection (data not shown).

The majority (64.5%) of subjects expressed no preference between injection locations after receiving all six injections, but if a preference was expressed (Table 3), the thigh was preferred (32.3% thigh, 3.2% abdomen).

## Depot localization

Ultrasound imaging qualified 42.3% of the depots as wholly within the targeted subcutaneous tissue (Figure 3). In 55.8% of injections, depots were observed to exhibit some partial intradermal infiltration above the predominantly subcutaneous depot (Figure 3). In select cases, higher volumes and/or viscosities combined with the effect of injection site and subject sex may have contributed to intradermal infiltration (87% frequency for 20 cP 10 ml abdominal injections in male subjects; 92% for 20 cP 5 ml thigh injections in female subjects); other injection conditions appear more equivalent between the sexes. Dual subcutaneous and intramuscular ($n = 2$) or intramuscular ($n = 1$, Figure 3) deposition was only observed for male subjects receiving 1.1 cP 5 ml thigh injections.

## In-line injection pressure

In-line injection pressure profiles were mapped per injection condition (Table 1) versus time (Figure 4). The injection pressure profiles demonstrate three phases: (1) initial rise at injection start, (2) a plateau-like region with a more gradual slope, and (3) decline after pump stops. Maximum injection pressure, time to maximum injection pressure, and area under the curve (AUC) were significantly different between solution viscosities ($p$ value ≤ 0.044) but not injection sites. Maximum injection pressure and AUC were significantly larger at increased viscosities and volumes ($p$ values ≤ 0.041).

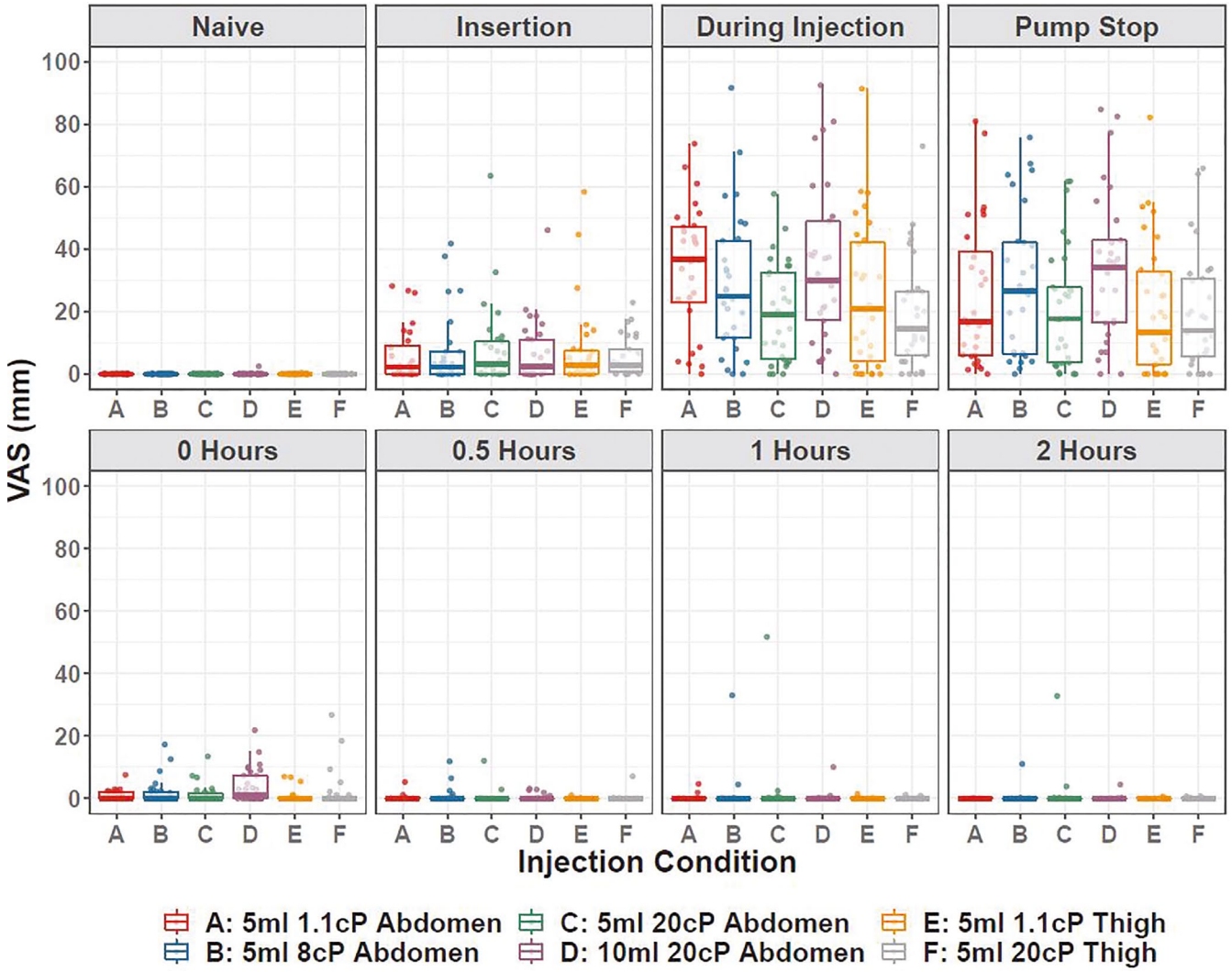


**FIGURE 2** Pain scores (visual analog scale [VAS] mm) over time for each injection condition. Boxplot displays median within first and third quartiles; the whiskers are 1.5 times the interquartile range (IQR). Dots are the individual data points. 0 hour = pump stop plus 10 min (device removal)

## DISCUSSION

The results of the clinical feasibility study demonstrate that large volume subcutaneous injections of 5 and 10 ml up to 20 cP in viscosity are both feasible and well-tolerated over an injection duration of 4.1 (5 ml) or 8.2 (10 ml) minutes in the abdomen and thigh. Favorable subject acceptability and tolerability combined with rapid resolution of tissue effects and pain across injection conditions suggest that the threshold of LVSC injections may exceed the traditional 1.5–3.0 ml limit and that the injection conditions tested may be within acceptable boundaries.[16–21]

Wheal formation and erythema post-injection were the most common observed tissue effects and likely due to the injection process and underlying LVSC deposition. Tissue effects resolved rapidly, although not completely, within the 2-hour in-clinic observation period of the study with few reported residual effects during the 24–72 hour follow-ups. Tissue effect data trends indicate more rapid resolution for abdominal and lower volume or viscosity injections, highlighting the potential for formulation-associated tissue effects and the value of further examination under specific formulations and conditions of use. Despite these variables, the current combination of VAS scores and Likert acceptability responses shows good potential for subject LVSC injection acceptability at 5 and 10 ml volumes over a broad range of delivery conditions, formulations, and delivery sites.

Several prior published studies show a limited impact of injection rate without clinically significant differences for subject VAS pain perception and others an observed trend toward lower VAS scores at slower rates.[17,21,30,35,37,41,42] LVSC administrations up to 600 ml have previously been well-tolerated when co-delivered with permeation enhancers, such as recombinant human hyaluronidase.[1,29] The current study used a single, constant, pump-driven

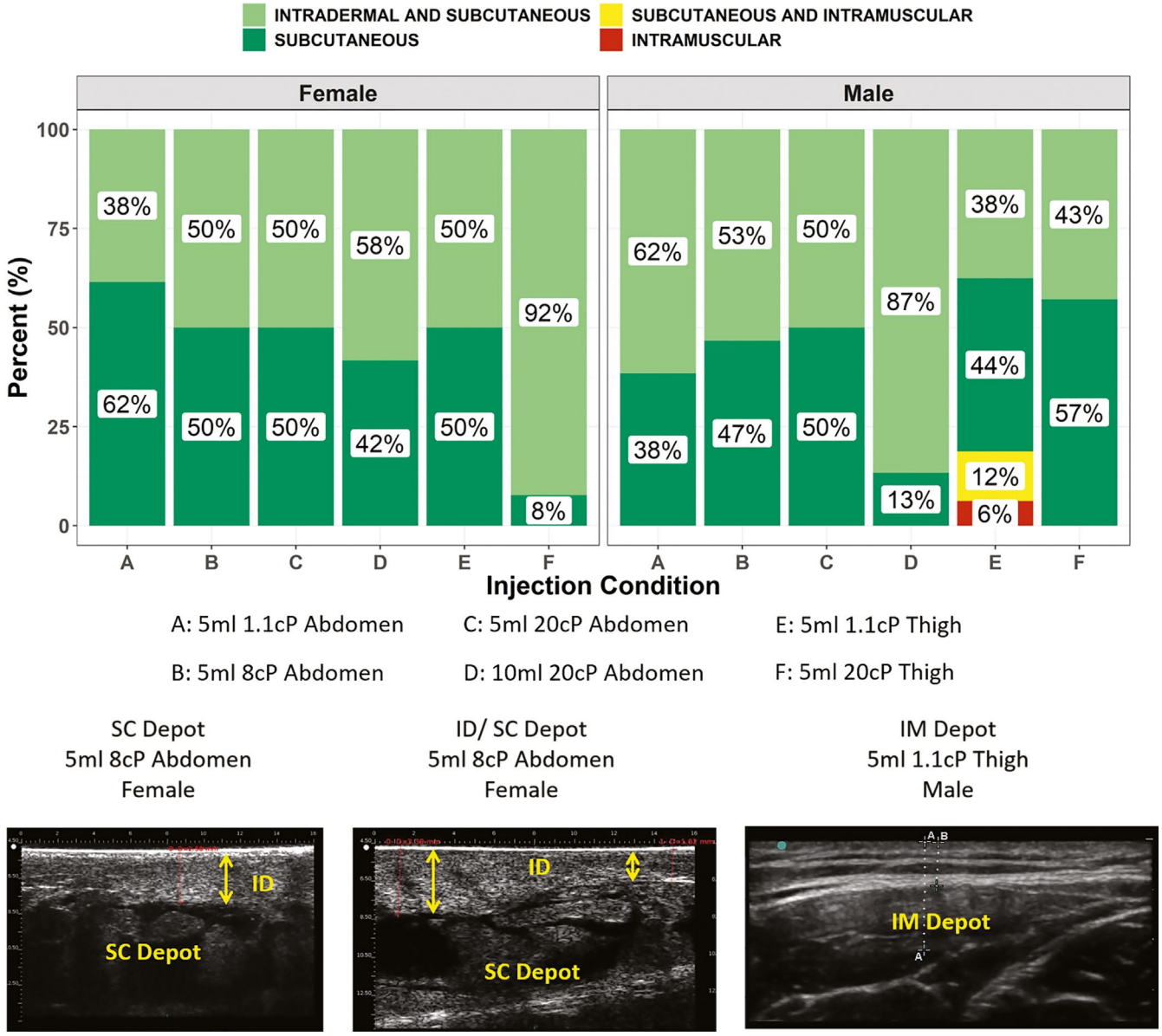


**FIGURE 3** Top: Percentage of depot locations in tissue layers (intradermal, subcutaneous, intramuscular, or combination) per injection condition and sex determined by qualitative assessment of ultrasound images. Bottom: Representative ultrasound images of subcutaneous (SC; entirely localized in subcutaneous), intradermal and subcutaneous (ID/SC; predominantly subcutaneous with minor intradermal infiltration), and intramuscular (IM) depots

delivery rate (20 μl/s) for both 5 and 10 ml volumes, selected to approximate a pertinent injection rate for a body-worn wearable injector.[27] Current and previously published VAS and corresponding acceptability results suggest that delivery periods over several minutes may allow for increased volume tolerability without the need for permeation enhancers.[27]

VAS pain scores peaked during and at delivery end with a broad distribution (Figure 2) per injection condition and no clinically significant differences (10 mm).[30,33–38] Data trends for the 5 ml abdominal injections suggest pain decreased as the viscosity increased (Table 2), a pattern also noted previously in similar injection studies.[30] The lowest viscosity solution, physiological saline, had the highest abdominal VAS scores and was used to dilute the hyaluronic acid for the 8 cP (10% HA, 90% saline) and 20 cP (20% HA, 80% saline) solutions. Although the VAS pain score pattern suggests decreased pain at increased viscosities, consideration of the specific formulation is also necessary. Current responses may be specific to clinical placebo (saline with or without HA). Injection pain may be highly influenced by the properties of individual formulations, such as active pharmaceutical ingredient, preservatives, diluent, pH, and osmolality.[21,35] Physiological saline may act as a mild irritant in the intradermal and subcutaneous tissue; therefore, the decrease in pain scores may reflect

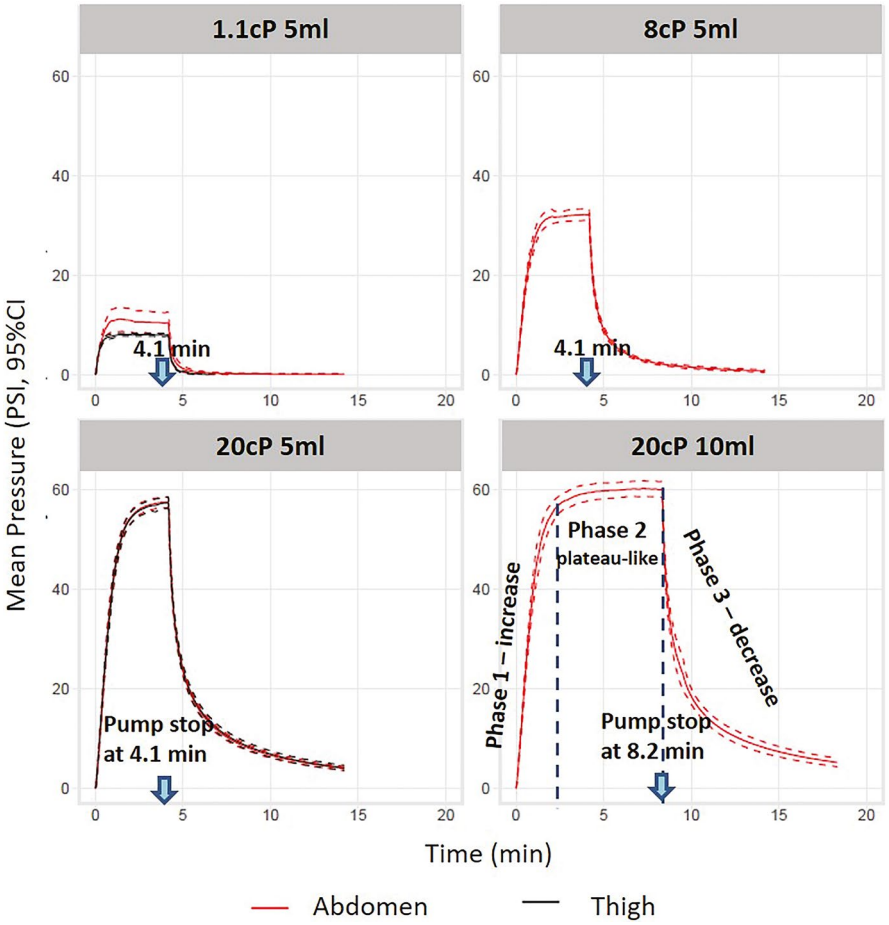


**FIGURE 4** In-line injection pressure curves for constant rate 20 μl/s injections into the human abdomen (red) or thigh (black). Each curve is the mean value of all included injections (Table 1) per delivery condition (solid line) with the ±95% confidence interval (CI) indicated by the dotted line. The blue arrows denote the time point at which the pump stopped: 4.1 min for 5 ml and 8.2 min for 10 ml. Three phases observed across the curves: (1) initial rise, (2) plateau-like region (gradual slope), and (3) decline after injection ceases

the dilution of saline as the HA is added and viscosity increases.[19,43–45]

VAS mean (34.3 mm, SD 20.2) and median (36.7 mm) pain score trends during injection appear more painful for abdominal 5 ml 1.1 cP (saline) injections than comparable thigh injections (mean 24.8 mm, SD 23.1, median 20.8); this is in contrast to previous studies in literature, which found the abdomen less painful.[21,35,41,42] Pain scores for 5 ml 20 cP (20% HA, 80% saline) abdomen (mean 19.6 mm, SD 16, median 19.1) and thigh (mean 19.7 mm, SD 18, median 14.4) injections are more equivalent, suggesting formulation and viscosity may contribute more to perceived pain than injection site. Despite pain score trends, most subjects had no preference (64.5%) between the injection sites, but if a preference was expressed, the thigh (32.3%) was selected over the abdomen (3.2%). Peak VAS score data trends during injection for 10 ml (mean 34.6 mm, SD 25.2) versus 5 ml 20 cP abdominal injections (mean 19.6 mm, SD 16) suggest increasing volume may also influence subject tolerability. The correlation between the VAS pain scores and tissue effects or injection pressure was weak (Pearson's rho ±0–04). The majority (86.2%–100%) of subject acceptability responses indicated no injection site pain, itching, burning, or soreness immediately post-injection across all injection conditions tested.

While VAS pain measurement has good reliability and correlation to other pain scores,[33] pain is a subjective experience that can exhibit variability amongst individuals.[17,42] Our designation of MCSD as 10 mm is consistent with published practice,[30,33–38] but no absolute consensus of the MCSD threshold is known. Current study results and others highlight the extensive number of factors that may impact VAS scores.[21,35,37,41,42] Additional studies assessing LVSC injections over an expanded range of volumes, viscosities, injection rates/durations, and varying formulations are needed to further extend and refine LVSC injection boundaries.

Deposition appeared entirely (42.3%) or predominantly (55.8%) localized within the target subcutaneous tissue in ultrasound images (Figure 3). Some ultrasound images showed localized infiltration of the overlying dermis (55.8%) that may be characterized by either visible ultrasound striations in the dermis and/or partial dermal thickening compared to the surrounding dermis (Figure 3). The ultrasound range may be insufficient to clearly distinguish depot lower margins or provide quantitative assessment of relative depot distribution in the current study, but the majority of the delivered dose appears localized within the subcutaneous tissue based on ultrasound specialist observation of characteristics such as visible fluid

deposition (e.g., lower reflectance) in the subcutaneous tissue. Additionally, widespread intradermal distension that would be expected from large intradermal injectate volumes was not observed. The rare (1.8%) instances of intramuscular infiltration ($n = 2$) or deposition ($n = 1$) occurred in three male subject 5 ml 1.1 cP thigh injections without apparent increase of VAS peak pain scores during the injection (0–20.8 mm) relative to all 5 ml 1.1 cP thigh injections overall (mean 24.8 mm, SD 23.1, median 20.8). Prior studies have demonstrated that males have lower average thigh subcutaneous tissue thickness.[46] Delivery into different tissues may alter pharmacokinetics (PKs)[16,24,47]; future PK studies are warranted when converting compounds from intravenous to LVSC dosing but were outside the scope of this trial.

The pump-driven system was optimized to a standard 10 minute equilibration interval between pump stop and device removal to allow pressure equilibration in the fluid path, maximize delivery volume equivalency (±20%) across solution viscosities, and maintain consistent clinical procedures. In-line injection pressures reflect contributions from both the delivery system and any tissue resistance that may occur during injection.[18,31,48,49] The pressure profiles obtained (Figure 4) show a three-phase profile with similar peak pressures, equilibration curves, and pressure declines per viscosity regardless of injection site location. Peak in-line delivery pressures increased with viscosity, as expected. Per fluid dynamic principles,[50] the extended tubing length and small internal diameter of the surrogate flow path may have contributed significantly to in-line peak pressures obtained. Body-worn devices for LVSC injection may have reduced fluid path length and/or altered drive mechanisms, delivery pressures, and rates[26,27]; each unique delivery system requires optimization and functional demonstration. Additionally, the HA placebo dilutions in this study are non-Newtonian; the impact of formulation rheological properties should also be considered for future LVSC system delivery optimization.

Subject recruitment in the current study was predominantly younger (mean 31.7 years, SD 9.8), normal BMI (mean 23.3 kg/m$^2$, SD 3.2), and Caucasian/White (30 of 32) subjects. A younger average subject age distribution and narrow demographics for some criteria were known limitations of this study. Inclusion of older subjects as well as a broader ethnicity and BMI distribution reflective of potential patient populations will be useful in establishing the impact of subject demographics and various morphologies on LVSC injection feasibility, acceptability, and tissue effects.

In conclusion, early clinical feasibility study results suggest viscous subcutaneous injections up to 20 cP and volumes of 10 ml in the abdomen or 5 ml in the thigh are clinically feasible and tolerable. Localized tissue effects and pain are transient with broadly favorable acceptability across all injection conditions. Results indicate that LVSC injection boundaries likely exceed both the traditional 1.5–3.0 ml limits and the conditions tested. Additional studies expanding on this early feasibility clinical including broader demographics and extended ranges of volumes, viscosities, flow rates, and formulations will further define LVSC injection boundaries and inform delivery system development.

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## CONFLICT OF INTEREST

W.D.W., D.R.M., D.E.S., R.J.P., and N.G.B. were employees of the study sponsor and funding entity, BD (Becton, Dickinson and Company), when the study was performed and may also be stockholders.

## AUTHOR CONTRIBUTIONS

W.D.W., D.R.M., D.E.S., R.J.P., and N.G.B. wrote the manuscript. W.D.W., D.E.S., R.J.P., and N.G.B. designed the research. W.D.W., N.G.B., and D.E.S. performed the research. W.D.W. and D.R.M. analyzed the data.

## DATA AVAILABILITY STATEMENT

The data are confidential and not publicly available.

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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