Original Article

# Pharmacokinetic and Pharmacodynamic Profile of Epinephrine Nasal Spray Versus Intramuscular Epinephrine Autoinjector in Healthy Adults

Matthew Greenhawt, MD, MBA, MSc[a,*], Jay Lieberman, MD[b,*], Michael Blaiss, MD[c], David I. Bernstein, MD[d], John Oppenheimer, MD[e], Lawrence DuBuske, MD[f], David Fleischer, MD[a], and David A. Dworaczyk, PhD[g] *Denver, Colo; Memphis, Tenn; Augusta, Ga; Cincinnati, Ohio; Newark and Lebanon, NJ; and Washington, DC*

***What is already known about this topic?*** There are limited comparative pharmacokinetic and pharmacodynamic data regarding novel intranasal versus conventional intramuscular (IM) methods of epinephrine delivery.

***What does this article add to our knowledge?*** The plasma epinephrine concentration was overall greater with epinephrine nasal spray versus IM autoinjector, with comparable effects on heart rate and blood pressure. Heart rate and blood pressure were weakly correlated with plasma epinephrine concentrations.

***How does this study impact current management guidelines?*** In this pooled analysis of 4 studies, the 13.2 mg epinephrine nasal spray in healthy adults resulted in similar or greater pharmacokinetic parameters and similar pharmacodynamics to an epinephrine IM autoinjector.

**BACKGROUND: Standard of care for anaphylaxis treatment is intramuscular (IM) epinephrine. An epinephrine nasal spray (ENS) is under development as an alternative form of administration.**

**OBJECTIVE: To compare the pharmacokinetic and pharmacodynamic (PD) profile of 13.2 mg ENS with 0.3 mg IM epinephrine autoinjector.**

**METHODS: Data from 4 open-label phase 1 crossover studies conducted in healthy adults were pooled to determine the pharmacokinetic and PD profile of a single 13.2 mg ENS dose delivered by 2 consecutive sprays of 6.6 mg each in opposite (n = 224 doses) or the same nostril (n = 75 doses) compared with the 0.3 mg IM autoinjector (n = 215 doses). Each participant served as their own control. Blood samples and vital**

[a]Section of Allergy and Immunology, Children's Hospital Colorado, Department of Pediatrics, University of Colorado School of Medicine, Denver, Colo
[b]The University of Tennessee Health Science Center, Memphis, Tenn
[c]Department of Pediatrics, Medical College of Georgia, Augusta, Ga
[d]Division of Immunology and Allergy, University of Cincinnati College of Medicine and Bernstein Clinical Research Center, Cincinnati, Ohio
[e]Department of Internal Medicine, University of Medicine and Dentistry of New Jersey-Rutgers New Jersey Medical School, Newark, NJ
[f]Department of Medicine, The George Washington University Hospital, Washington, DC
[g]Bryn Pharma, LLC, Lebanon, NJ
*Co-first authors.

Funding for this study was provided by Bryn Pharma.

Conflicts of interest: D.I. Bernstein is a consultant for Aquestive, ARS, and Bryn Pharma and has received research funding from ARS, Amgen, AstraZeneca, Cheisi, GlaxoSmithKline, Elodi, Novartis, Sanofi, and TEVA. M. Blaiss is a consultant for ALK-Abelló, AstraZeneca, GlaxoSmithKline, Hippo Dx, Prollergy, Regeneron, Sanofi, ARS, and Bryn Pharma. L. DuBuske has served on an advisory board and/or as a speaker for Bryn, GlaxoSmithKline, Regeneron, Sanofi, Amgen, and AstraZeneca, is a consultant for Allergy Therapeutics, Areteia, and ALK-Abelló, and has received royalties from UpToDate. D. Fleischer has received research support from ARS and DBV Technologies, serves as an unpaid advisory board member for Food Allergy & Anaphylaxis Connection Team and the National Peanut Board, receives royalties from UpToDate, is a member of physician/medical advisory boards to Aquestive, ARS, Bryn Pharma, DBV Technologies, Genentech, and Nasus, and is a speaker for Genentech. M. Greenhawt is a consultant for Aquestive, a speaker for Genentech, is a member of physician/medical advisory boards for DBV Technologies, Takeda, Nutricia, Novartis, Aquestive, Allergy Therapeutics, AstraZeneca, ALK-Abelló, Bryn Pharma, Genentech, and Prota, is an unpaid member of the Scientific Advisory Council for the National Peanut Board and the Medical Advisory Board of the International Food Protein Induced Enterocolitis Syndrome Association, is a member of the Brighton Collaboration Criteria Vaccine Anaphylaxis 2.0 Working Group, is the senior associate editor for the *Annals of Allergy, Asthma, and Immunology*, is a member of the Joint Taskforce on Allergy Practice Parameters, and has received honorarium for lectures from ImSci, Red Nucleus, Medscape, Paradigm Medical Communications, Kaplan, Food Allergy Research and Education, and multiple state/local allergy societies. J. Lieberman has received research funding (to institution) from DBV and Novartis, served as a consultant or advisor to Aquestive, ARS, Bryn Pharma, ALK, Genentech, and Novartis, and served as an adjudicator for Abbvie and Celldex. J. Oppenheimer has served as a consultant for ARS, Aquestive, Bryn, GlaxoSmithKline, and Sanofi and has served on adjudication or data safety monitoring boards for AstraZeneca, Amgen, GlaxoSmithKline, Novartis, Sanofi, and Regeneron. D. A. Dworaczyk was an employee of Bryn Pharma at the time of the study and currently provides consulting services for Bryn Pharma.

The studies included in this analysis were phase I studies and did not require trial registration.

Received for publication July 1, 2024; revised September 23, 2024; accepted for publication October 2, 2024.
Available online ■■

Corresponding author: Matthew Greenhawt, MD, MBA, MSc, Section of Allergy and Immunology, Children's Hospital Colorado, University of Colorado School of Medicine, 13123 East 16th Ave, Box 518, Anschutz Medical Campus, Aurora, CO 80045. E-mail: Matthew.Greenhawt@childrenscolorado.org.

2213-2198



https://doi.org/10.1016/j.jaip.2024.10.006

*Abbreviations used*
*AUC- area under the curve*
*BMI- body mass index*
*BP- blood pressure*
*$C_{max}$- maximum observed plasma concentration*
*DBP- diastolic blood pressure*
*ENS- epinephrine nasal spray*
*HR- heart rate*
*IM- intramuscular*
*PD- pharmacodynamic*
*PK- pharmacokinetic*
*SBP- systolic blood pressure*
*$T_{max}$- time to reach $C_{max}$*

**signs were collected predose and at multiple intervals from 0 to 360 minutes postdose.**
**RESULTS: ENS rapidly increased the plasma epinephrine concentration, with levels that were overall greater than IM autoinjector. Median (range) time to maximum plasma epinephrine concentration with ENS opposite nostrils, ENS same nostril, and IM autoinjector was 25.1 (1.3-362.1), 20.1 (3.0-120.2), and 20.0 (1.0-121.3) minutes, respectively. The area under the plasma concentration−time curve for 0 to 360 minutes was significantly higher with ENS than with the IM autoinjector (geometric mean ratio [90% CI], 155% [140%-172%] with ENS opposite nostrils, 159% [138%-182%] with ENS same nostril). The PD effects on heart rate and blood pressure were similar in pattern and magnitude among all 3 treatment groups.**
**CONCLUSIONS: ENS rapidly achieved plasma epinephrine levels greater and more sustained than the IM autoinjector and with a similar PD effect.**  **(J Allergy Clin Immunol Pract 2024;■:■-■)**

***Key words:*** *Anaphylaxis; Epinephrine; Intranasal; Pharmacodynamics; Pharmacokinetics*

## INTRODUCTION

Anaphylaxis is a serious immediate allergic reaction to food, insect stings, medications, and/or other allergens that requires urgent treatment intervention to avoid morbidity and potentially even fatality.[1] Epinephrine administered intramuscularly (IM) is the standard of care for treating anaphylaxis because oral administration results in very low bioavailability.[2] Epinephrine is a sympathomimetic α-adrenergic as well as a β1- and β2-adrenergic agonist. The pharmacodynamic (PD) effects of epinephrine include increased heart rate (HR) and changes in blood pressure (BP).[3]

Patients at a higher risk of anaphylaxis are often prescribed epinephrine IM autoinjectors because the vast majority of anaphylactic events occur at home or in a community setting.[2] However, epinephrine self-administration via autoinjectors for anaphylaxis is suboptimal because of lack of carriage[4] and needle-phobia, which may result in delayed administration or failure to use epinephrine at all.[5,6] In a study of 190 patients prescribed epinephrine IM autoinjectors, only 30% of those who experienced an anaphylactic event actually used it.[6] Delays or failure to self-administer epinephrine increase the risk of anaphylaxis-related hospitalization and mortality.[7-9]

Nasally administered epinephrine products are under development as an alternative method of epinephrine self-administration for the treatment of anaphylaxis. Clinical trials to compare the efficacy of epinephrine nasal spray (ENS) with an epinephrine IM autoinjector in treating patients with anaphylaxis are not deemed to be ethical. Instead, regulatory authorities rely on pharmacokinetic (PK) and PD studies that compare bioavailability, absorption, and PD effects of novel noninjectable epinephrine products with IM administration. Thus, the objective of this analysis was to compare the PK and PD profile of 13.2 mg ENS with that of the standard of care, 0.3 mg IM epinephrine autoinjector, using pooled data from 4 studies.

## METHODS

Data from 4 open-label phase 1 crossover studies of ENS (NDS1C, Bryn Pharma, Lebanon, NJ) were pooled for PK and PD analysis.[10-13] All studies were approved by an institutional review board (Advarra, Columbia, Md), and written informed consent was obtained from all participants. The studies were conducted in compliance with the principles of the Declaration of Helsinki and Good Clinical Practice.

Participants in all 4 studies were healthy, nonsmoking adults aged 19 to 65 years with a body mass index (BMI) of greater than or equal to 18.0 and less than or equal to 32.0 kg/m$^2$ at screening. "Healthy" was defined as no clinically significant medical history, physical exam, laboratory profiles, vital signs, or electrocardiograms, per the judgment of the study investigator or designee. Participants were not experiencing anaphylaxis during the conduct of the studies. The pooled treatment arms were a single 13.2 mg ENS dose delivered by 2 consecutive sprays of 6.6 mg each in opposite nostrils, a single 13.2 mg ENS dose delivered by 2 consecutive sprays of 6.6 mg each in the same nostril, and a single 0.3 mg epinephrine dose delivered by IM autoinjector (Mylan Specialty L.P., Morgantown, Wva). The single dose is administered as 2 sprays because a previous dose-ranging study demonstrated that the 13.2 mg intranasal dose, delivered as 2 sprays of 6.6 mg each to opposite nostrils, produced a PK profile and plasma epinephrine level comparable to the 0.3 mg dose by IM autoinjector and the 0.5 mg dose by IM manual syringe.[14] The consecutive intranasal sprays were administered within no more than 10 seconds of each other. The IM autoinjector injections were administered to the middle of the outer thigh. All treatments were administered by trained clinical personnel, except in one treatment period in one of the studies the ENS dose was self-administered.

In all studies, each participant served as their own control per the crossover designs, with a washout period of at least 1 day between ENS and IM autoinjector treatment periods and of at least 14 days between the ENS treatment periods.

### PK and PD analysis

Blood samples to measure plasma epinephrine concentrations and HR, systolic blood pressure (SBP), and diastolic blood pressure (DBP) as indicators of a PD effect were collected or measured at −30, −20, −10 minutes predose and 1, 3, 5, 7, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180, and 360 minutes postdose.

Plasma concentrations of epinephrine were determined using a validated ultraperformance liquid chromatographic method with a tandem mass spectrometry detection method. The analytical range was 15 to 2000 pg/mL. PK parameters included area under the plasma concentration−time curve (AUC) from time 0 to the 10-,

20-, 30-, 60-, and 360-minute postdose time points ($AUC_{0\text{-}10}$, $AUC_{0\text{-}20}$, $AUC_{0\text{-}30}$, $AUC_{0\text{-}60}$, and $AUC_{0\text{-}360}$), the maximum observed concentration ($C_{max}$), $C_{max}$ from time 0 to 20 minutes, and time to reach $C_{max}$($T_{max}$). The percentage of participants attaining baseline-adjusted plasma epinephrine concentrations of 50, 100, and 200 pg/mL at 10, 20, 30, and 60 minutes postdose was calculated for each treatment arm.

### Statistical analysis

Differences in demographic characteristics among the 4 study populations were evaluated by rank-sum test for continuous variables and Fisher exact test or $\chi^2$ test for categorical variables. All available participant-level data for each baseline-adjusted PK or PD parameter were pooled by treatment arm. An average of 3 predose measurements for plasma epinephrine concentration, HR, and BP were used for baseline adjustments for each participant. Summary statistics for PK and PD parameters were calculated by treatment arm and time point. An ANOVA was performed on the natural log-transformed AUC and $C_{max}$ plasma epinephrine parameters for each treatment arm. Geometric least-squares means were calculated using the exponentiation of the least-squares means from the ANOVA. Test-to-reference ratios of the geometric least-squares mean and corresponding 90% CIs were calculated as the difference between test and reference values and expressed as a percentage relative to the reference. 90% CI values that do not cross 100% are considered statistically significant. Correlations between baseline-adjusted plasma epinephrine concentrations and unadjusted HR, SBP, and DBP after administration of ENS (combined opposite nostril and same nostril treatment arms) were analyzed by Spearman correlation coefficient ($\rho$) for 10-, 20-, and 360-minute postdose values. Values for IM autoinjector were not included in the analysis. Epinephrine concentrations of less than 10 or more than 1000 pg/mL were excluded from the analysis. A correlation between BMI and unadjusted $C_{max}$ with 13.2 mg ENS in opposite nostrils was assessed with Spearman correlation coefficient, and the effect of sex on unadjusted $C_{max}$ with 13.2 mg ENS in opposite nostrils was assessed with nonparametric Mann-Whitney test. Multivariate analysis controlling for covariates of epinephrine concentration, age, and race was used with the pooled data to determine an association between BMI and sex with unadjusted HR. $P$ values less than .05 were considered statistically significant.

## RESULTS

### Participants

There were no significant differences in demographic characteristics among the individual study populations (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). In the pooled population, 53% were male, 63% identified as White, 24% identified as Black, and the mean age was 40 years (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). In all, 251 participants were randomized in the 4 studies; per the crossover study designs, 176 participants were randomized to 224 doses of 13.2 mg ENS in opposite nostrils, 75 participants were randomized to 75 doses of 13.2 mg ENS in the same nostril, and 215 participants were randomized to 215 doses of 0.3 mg epinephrine by IM autoinjector.

### PK data

Administration of ENS resulted in a rapid increase in plasma epinephrine concentration, with exposure that was overall greater than IM autoinjector (Figure 1). The plasma epinephrine concentration was higher between 5 and 10 minutes with the IM autoinjector than with ENS (Figure 1). The median (range) time to reach $C_{max}$ (minutes) with ENS in opposite nostrils, ENS in the same nostril, and IM autoinjector was 25.1 (1.3-362.1), 20.1 (3.0-120.2), and 20.0 (1.0-121.3), respectively (Table I). The baseline-adjusted geometric means for $AUC_{0\text{-}10}$, $AUC_{0\text{-}20}$, $AUC_{0\text{-}30}$, $AUC_{0\text{-}60}$, and $C_{max}$ were generally similar between ENS in either opposite nostrils or the same nostril compared with the IM autoinjector (Table I and Table II). The baseline-adjusted geometric mean $AUC_{0\text{-}360}$ was significantly higher with ENS than with the IM autoinjector, with a geometric mean ratio (90% CI) of 155% (140%-172%) with ENS in opposite nostrils and 159% (138%-182%) with ENS in the same nostril compared with the IM autoinjector (Table II). The proportion of participants attaining specific concentration thresholds of 50, 100, and 200 pg/mL at 10 to 60 minutes postdose was generally similar across all treatments, although there was a trend toward ENS in opposite nostrils having smaller proportions of participants reaching the thresholds compared with the ENS same nostril and IM autoinjector groups (Figure 2). There was no statistically significant correlation with ENS between BMI and $C_{max}$ ($\rho = 0.09$; $P = .19$) and no statistically significant difference in $C_{max}$ between males and females ($P = .37$; see Figure E1 in this article's Online Repository at www.jaci-inpractice.org).

### PD data

The PD effect on HR was similar in pattern and magnitude among all 3 treatment groups (Figure 3). The transient mean changes from baseline in HR and BP for all 3 treatment groups were negligible and not clinically concerning. The greatest mean increase from baseline in HR was 6.5 bpm with ENS in opposite nostrils (at 1-minute postdose), 8.8 bpm with ENS in the same nostril (at 45 minutes postdose), and 5.9 bpm with the IM autoinjector (at 5 minutes postdose). After controlling for other covariates, the independent variables of BMI and sex were not significantly predictive of change in HR. The effect on SBP and DBP was also similar in pattern and magnitude among all 3 treatment groups (Figure 4). The small observed mean decrease in SBP and DBP in all 3 treatment groups was less than or equal to 4.3 mm Hg from baseline at any time point. A plateau in HR and BP was reached in all treatment groups (Figures 3 and 4). Although there was interparticipant variability in the PD effects, total postdose median and mean values for HR, SPB, and DBP were not significantly different between ENS and the IM autoinjector (Figure 5).

### Correlation between PK and PD

There was a weakly significant correlation between the plasma epinephrine concentration and HR at the analyzed time points of 10, 20, and 360 minutes postdose after ENS ($\rho \leq 0.32$, all $P < .0001$; Table III). There was no significant correlation between epinephrine concentration and SBP at any analyzed time point ($P \geq .16$), whereas there was a weak but statistically significant negative correlation between epinephrine concentration and DBP at 20 and 360 minutes postdose ($r \geq -0.12$, both $P = .04$) but not at 10 minutes postdose (Table III).

## DISCUSSION

In this pooled analysis of studies conducted in healthy adults, a single cumulative dose of 13.2 mg ENS delivered in opposite nostrils or the same nostril achieved comparable time to reach $C_{max}$ and $C_{max}$ to the 0.3 mg IM autoinjector. Concentrations of

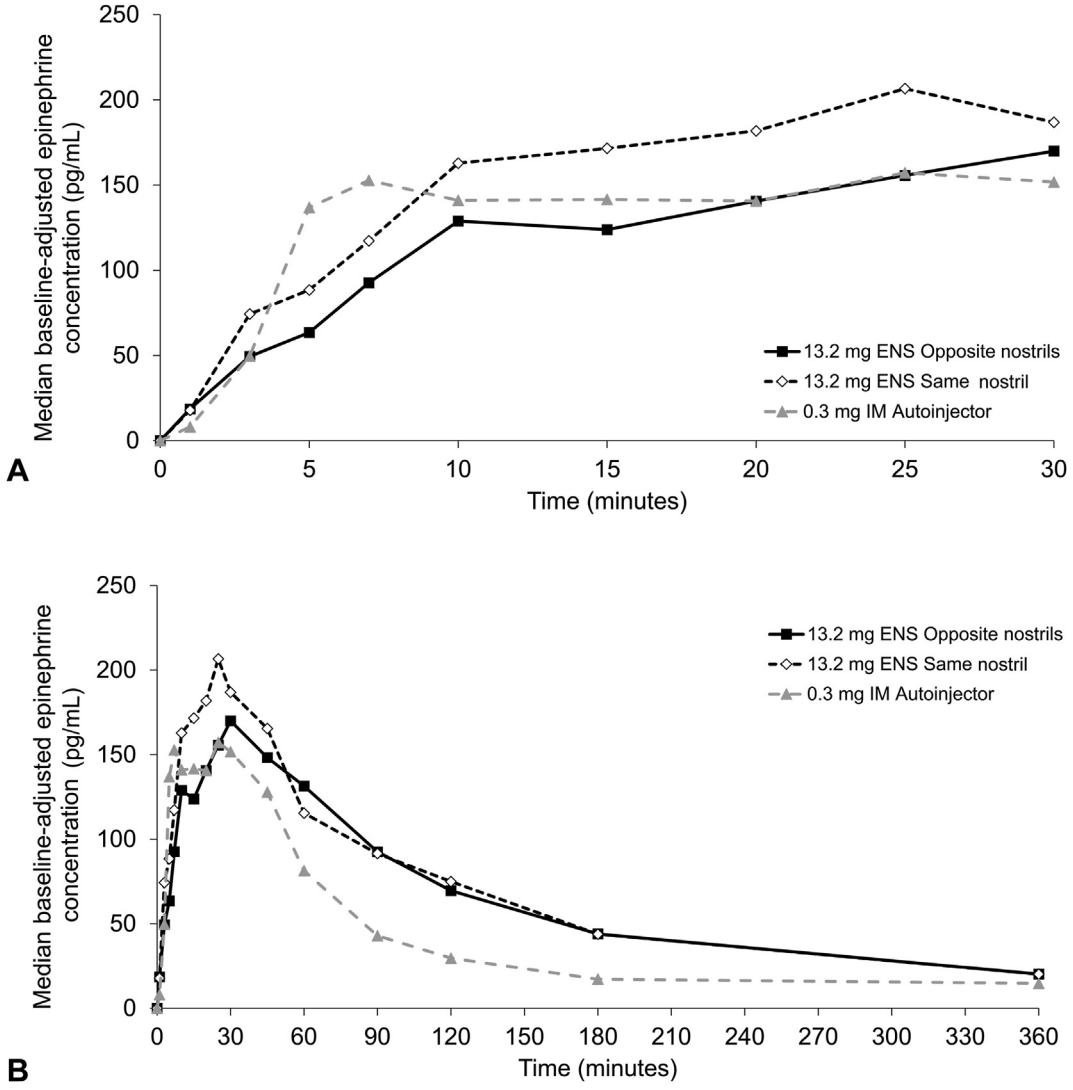


**FIGURE 1.** Median baseline-adjusted plasma epinephrine concentration—time profiles from (**A**) 0 to 30 minutes and (**B**) 0 to 360 minutes.

**TABLE I.** Baseline-adjusted plasma epinephrine PK outcomes

| PK Parameter | 13.2 mg ENS in opposite nostrils (n = 224*) | 13.2 mg ENS in the same nostril (n = 75*) | 0.3 mg IM autoinjector (n = 215*) |
|---|---|---|---|
| $AUC_{0\text{-}10}$ (pg • min/mL), geometric mean (CV%) | 603 (326) | 861 (166) | 942 (155) |
| $AUC_{0\text{-}20}$, (pg • min/mL), geometric mean (CV%) | 2,002 (186) | 2,741 (109) | 2,370 (104) |
| $AUC_{0\text{-}30}$, (pg • min/mL), geometric mean (CV%) | 3,879 (134) | 4,856 (97) | 4,072 (83) |
| $AUC_{0\text{-}60}$, (pg • min/mL), geometric mean (CV%) | 8,953 (115) | 10,240 (84) | 8,217 (65) |
| $AUC_{0\text{-}360}$, (pg • min/mL), geometric mean (CV%) | 27,130 (92) | 27,710 (75) | 17,480 (52) |
| $C_{max0\text{-}20}$ (pg/mL), geometric mean (CV%) | 191.4 (151.9) | 257.3 (99.6) | 226.9 (103.3) |
| $C_{max}$ (pg/mL), geometric mean (CV%) | 262.8 (114.4) | 332.0 (82.0) | 285.7 (76.4) |
| $T_{max}$ (min), median (minimum, maximum) | 25.1 (1.3, 362.1) | 20.1 (3.0, 120.2) | 20.0 (1.0, 121.3) |

*$AUC_{0\text{-}x}$*, Area under the curve from 0 to *x* minutes postdose; *$C_{max20}$*, maximum observed concentration from 0 to 20 minutes; *CV*, coefficient of variation; *$T_{max}$*, time to reach maximum concentration.
n is the number of randomized doses.

**TABLE II.** Comparison of baseline-adjusted plasma epinephrine PK parameters

| PK Parameter | 13.2 mg ENS in opposite nostrils (n = 224*) Geometric LSM | 0.3 mg IM autoinjector (n = 215*) Geometric LSM | Geometric mean ratio, % | 90% CI | Intrasubject CV% |
|---|---|---|---|---|---|
| $AUC_{0\text{-}10}$ (pg • min/mL) | 603 | 942 | 64 | 51-80 | 216 |
| $AUC_{0\text{-}20}$ (pg • min/mL) | 2,002 | 2,370 | 85 | 71-100 | 133 |
| $AUC_{0\text{-}30}$ (pg • min/mL) | 3,879 | 4,072 | 95 | 83-110 | 103 |
| $AUC_{0\text{-}60}$ (pg • min/mL) | 8,953 | 8,217 | 109 | 97-123 | 82 |
| $AUC_{0\text{-}360}$ (pg • min/mL) | 27,130 | 17,480 | 155 | 140-172 | 66 |
| $C_{max}$ (pg/mL) | 262.8 | 285.7 | 92 | 81.3-104.0 | 86 |
| | **13.2 mg ENS in the same nostril (n = 75*)** | **0.3 mg IM autoinjector (n = 215*)** | | | |
| $AUC_{0\text{-}10}$ (pg • min/mL) | 861 | 942 | 91 | 68-123 | 216 |
| $AUC_{0\text{-}20}$ (pg • min/mL) | 2,741 | 2,370 | 116 | 92-145 | 133 |
| $AUC_{0\text{-}30}$ (pg • min/mL) | 4,856 | 4,072 | 119 | 98-144 | 103 |
| $AUC_{0\text{-}60}$ (pg • min/mL) | 10,240 | 8,217 | 125 | 106-146 | 82 |
| $AUC_{0\text{-}360}$ (pg • min/mL) | 27,710 | 17,480 | 159 | 138-182 | 66 |
| $C_{max}$ (pg/mL) | 332.0 | 285.7 | 116.2 | 98.3-137.2 | 85.6 |

*$AUC_{0\text{-}x}$*, Area under the curve from 0 to *x* minutes postdose; *CV*, coefficient of variation; *LSM*, least-squares means.
n is the number of randomized doses.

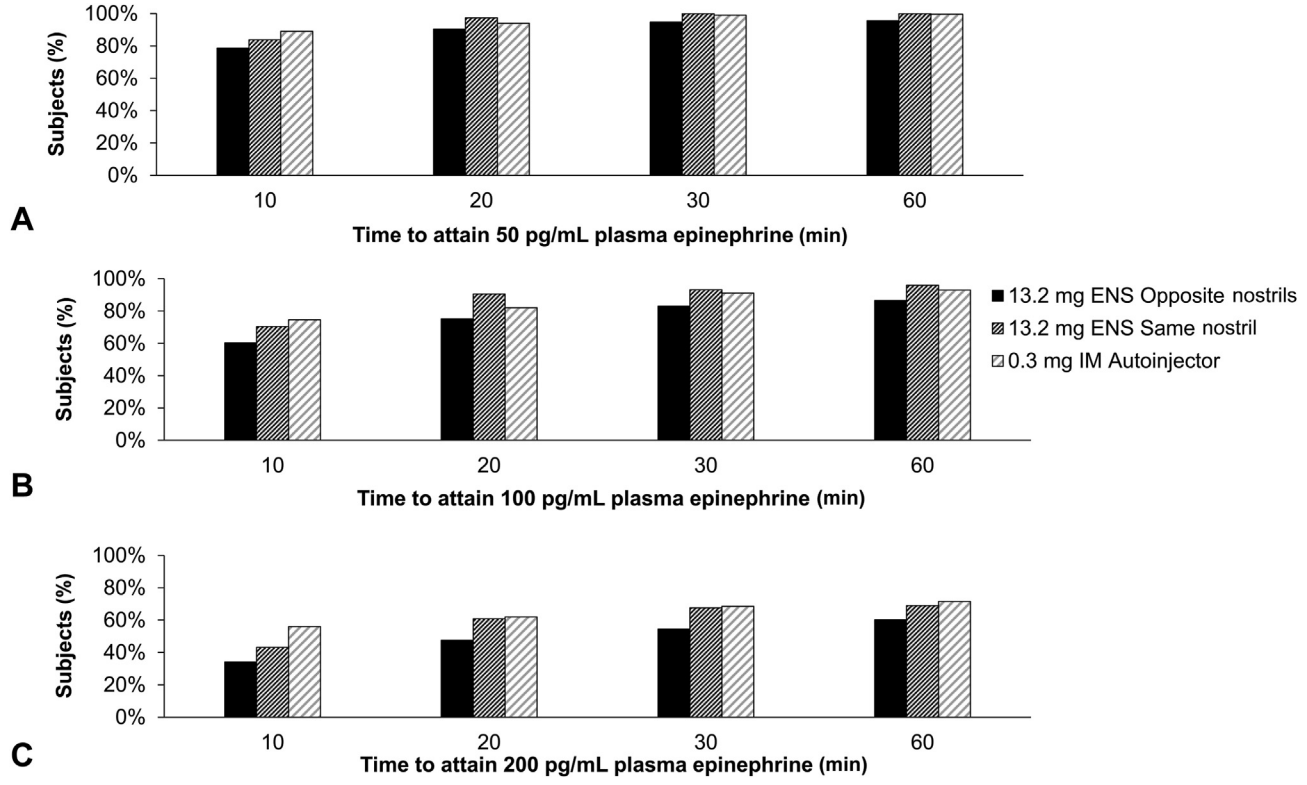


**FIGURE 2.** Proportion of participants attaining baseline-adjusted plasma epinephrine concentrations of (**A**) 50 pg/mL, (**B**) 100 pg/mL, and (**C**) 200 pg/mL.

plasma epinephrine were, overall, greater with 13.2 mg ENS over the 6-hour time course ($AUC_{0\text{-}360}$) than the IM autoinjector, but with similar PD effects. The plasma epinephrine concentrations were maintained for longer with ENS compared with the IM autoinjector.

The ENS is under development as the current IM epinephrine administration presents several concerns. A challenge from a clinical aspect is that the bioavailability of an IM delivered dose is dependent on the patient's skin-to-muscle distance and can also vary among autoinjector brands because of the device's

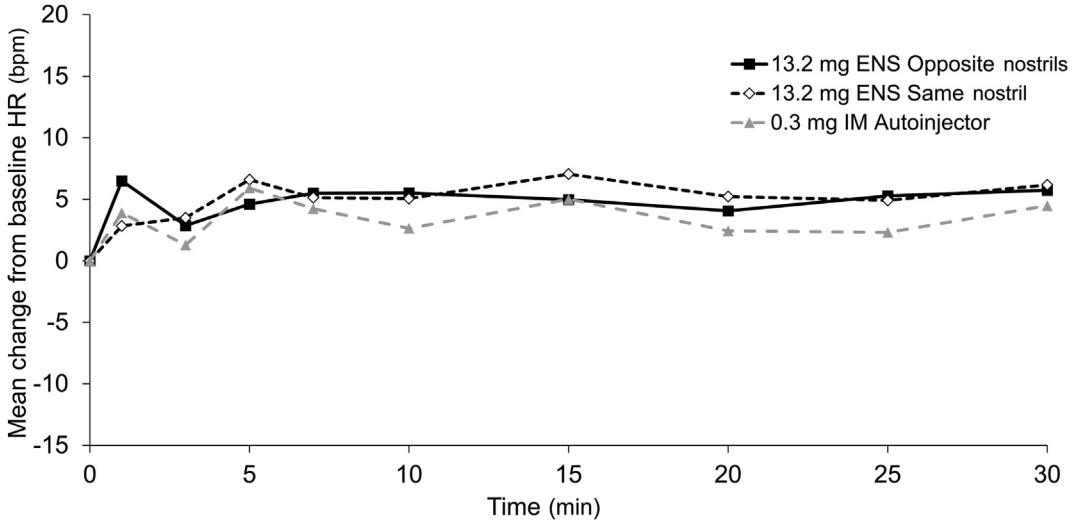


**FIGURE 3.** Mean change from baseline HR-time profiles.

functional properties (ie, device mechanism used).[15] Furthermore, the patient's skin-to-muscle distance and use of the autoinjector through clothing could possibly result in subcutaneous delivery instead of IM delivery. From a psychological aspect, some patients or caregivers may fear using the IM autoinjector because they are concerned the injection itself may cause an injury that is more harmful than the anaphylaxis.[5,16] Thus, because of device variability among autoinjectors and poor patient adherence, an alternative method of epinephrine delivery is a pressing unmet need for the treatment of anaphylaxis.[17]

Rapid absorption of epinephrine is critical to successful anaphylaxis treatment because anaphylaxis can rapidly progress. This analysis found that absorption of epinephrine was indeed rapid when delivered by the nasal spray; plasma epinephrine was detected within minutes of administration, and by 10 minutes postdose, most participants had already attained the Food and Drug Administration—required benchmark concentration of 100 pg/mL.

The PD effects on HR and BP were similar between ENS and IM administration, although the large number of outliers outside the interquartile analysis indicates high interparticipant variability in PD effects in all treatment arms, including the current standard of IM epinephrine. After ENS administration there was a weak correlation between plasma epinephrine concentrations and HR (positive correlation) and DBP (negative correlation) and no correlation with SBP when examining early (10 or 20 minutes) and late (360 minute) time points. In a pooled analysis of 4 PK/PD trials with another ENS, the effects on HR and SBP were comparable between the ENS and IM administration, whereas DBP was increased with the ENS and decreased with IM administration.[18] In the same pooled analysis, a relationship between plasma epinephrine concentrations and HR and SBP was observed until the epinephrine concentration reached 500 pg/mL, and then SBP plateaued; no clear relationship between epinephrine concentrations and DBP after ENS administration was observed.[18] A review of PK/PD studies reported conflicting PD effects among various IM autoinjectors, with a clear relationship between plasma epinephrine levels and HR and BP with some autoinjectors whereas an inconclusive relationship was seen with other autoinjectors.[15] Together, the data are in agreement that plasma epinephrine positively correlates with a modest, transient increase in HR. However, the disparate findings concerning a correlation between plasma epinephrine levels and BP are somewhat perplexing considering that the mechanism of action during anaphylaxis is to increase cardiac output and increase peripheral resistance, thereby counteracting hypotension. The PD effects of epinephrine may be impacted by the physiological processes that occur in patients experiencing an anaphylactic reaction that are not being experienced in healthy adults and warrant further investigation.

A limitation of this analysis is that the plasma epinephrine concentration needed to treat anaphylaxis is not known because PK studies have only been conducted in healthy individuals. The standard required by the Food and Drug Administration for approval of epinephrine products, no matter which device is studied, is comparability of changes in PK and PD parameters being induced by the administration of epinephrine in healthy subjects. Using this standard, IM administration in the thigh became preferred in anaphylaxis treatment guidelines over subcutaneous administration when it was demonstrated that IM administration in the thigh achieved a more rapid and sustained impact on PK parameters.[19,20] Similarly, novel epinephrine autoinjector devices have been approved by the Food and Drug Administration after demonstration of comparable PK and PD parameters with those achieved by a conventional autoinjector device.[21] The current analysis of pooled data indicates that administration of 13.2 mg ENS achieved the plasma epinephrine levels of the reference standard (eg, IM autoinjector administration), suggesting comparative therapeutic levels were reached. Another limitation of the analysis is that the mean BMI was 26.8, indicating an overweight population. Needle penetration of IM injections can vary by BMI,[22] with the potential for decreased plasma epinephrine levels in the obese population because of a lack of penetration of the vastus lateralis. Individuals with BMI more than 32 $kg/m^2$ were not eligible for the trial to minimize the possibility of subcutaneous injections. Moreover, all the IM injections were administered by trained personnel to try and

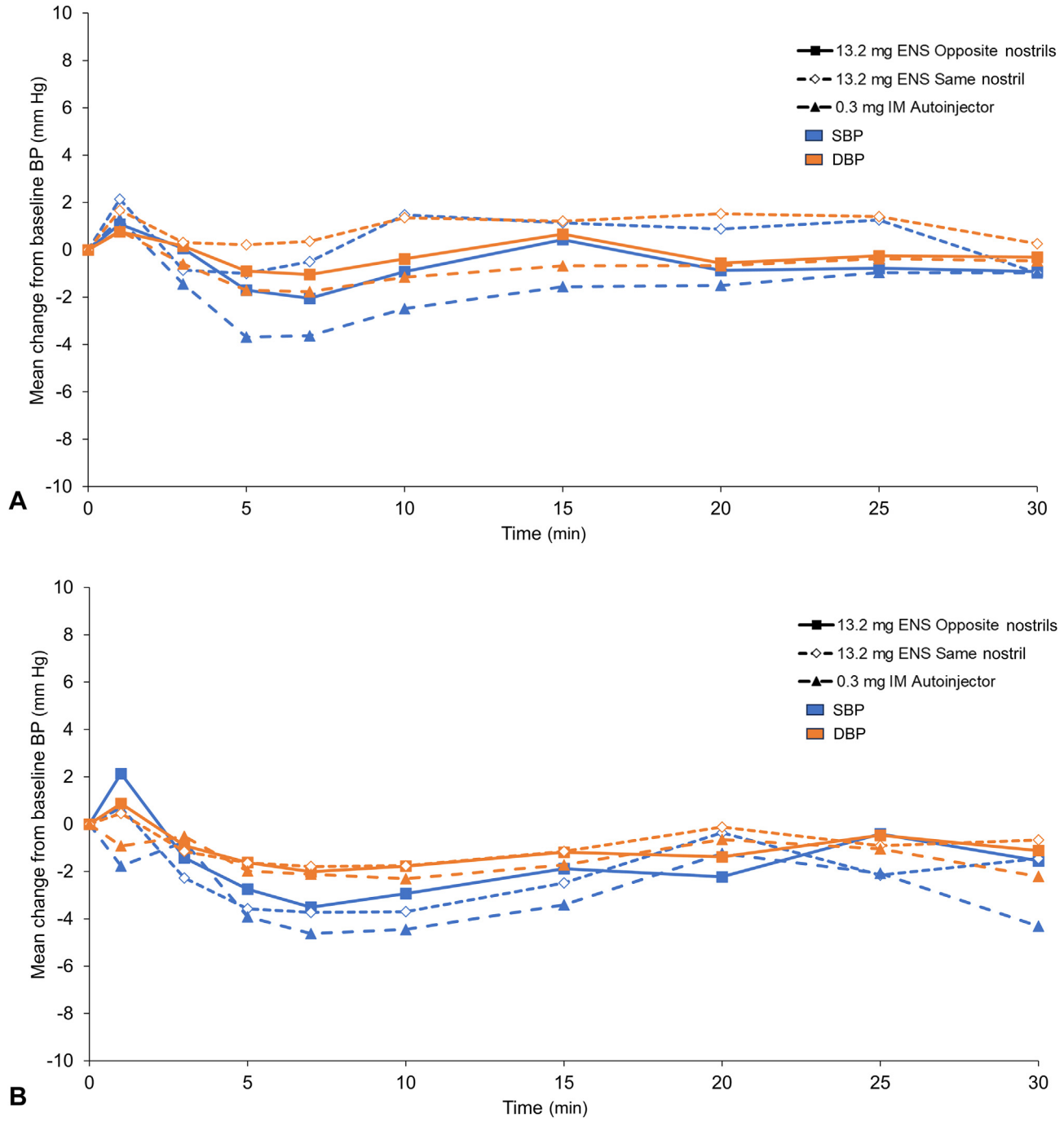


**FIGURE 4.** Mean change from baseline BP-time profiles in (**A**) males and (**B**) females.

ensure proper IM injection. However, there were no procedures, such as ultrasound, to ensure that injections were IM and not subcutaneous. The open-label design of the studies with no placebo control is a limitation in regard to the PD effects because the blood sample collection itself can induce small HR and BP effects.[23] The PD measures were limited to only HR and BP; other measures such as mean arterial pressure could be explored as additional indicators of PD effects. Another limitation of the analysis is the small sample size of the ENS same nostril treatment arm, which is because this method of administration was evaluated in only 2 of the 4 included studies.

The challenges of IM autoinjectors in treating anaphylaxis highlight a need for alternative options for epinephrine administration. This pooled analysis indicates that 13.2 mg ENS delivered in opposite nostrils or the same nostril can result in plasma epinephrine levels that are similar or greater than with an IM autoinjector and with comparable PD effects. Like IM

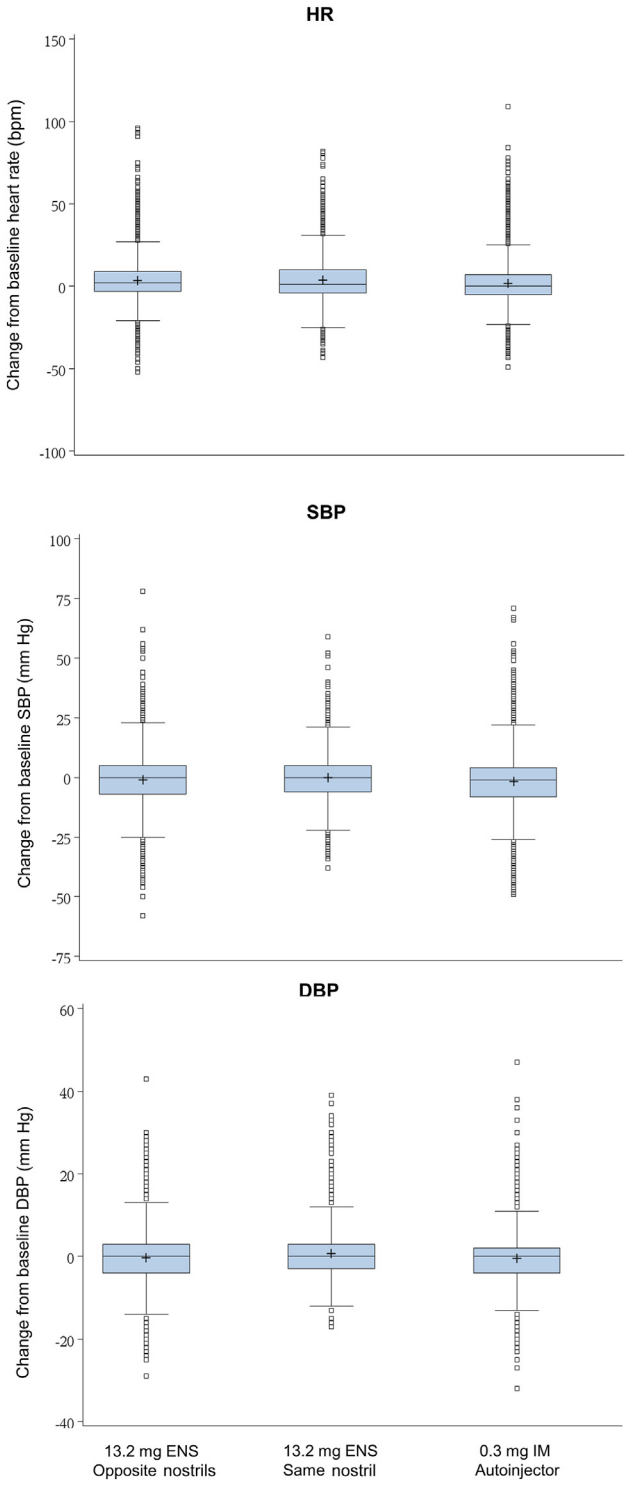


**FIGURE 5.** Summary values for change from baseline in HR, SBP, and DBP. The solid line within the box represents the median of all individual participant values across all time points, and the "+" represents the mean. Upper and lower whiskers represent the largest and smallest observed values within 1.5× the interquartile range from the upper (Q3) and lower (Q1) quartiles. Square symbols are individual values outside the bounds of the whiskers.

**TABLE III.** Spearman correlation between plasma epinephrine concentration and PD effects after ENS administration

| PD parameter, time point (min) | Correlation vs plasma epinephrine concentration, $\rho$ | *P* value |
|---|---|---|
| HR | | |
| 10 | 0.32 | <.0001 |
| 20 | 0.29 | <.0001 |
| 360 | 0.29 | <.0001 |
| SBP | | |
| 10 | −0.07 | .24 |
| 20 | −0.08 | .16 |
| 360 | −0.08 | .16 |
| DBP | | |
| 10 | −0.05 | .36 |
| 20 | −0.12 | .04 |
| 360 | −0.12 | .04 |

epinephrine, the response is rapid for the timely treatment of anaphylaxis, making ENS a viable alternative option to IM epinephrine delivery.

## Acknowledgments

This research was supported by Bryn Pharma, LLC. We acknowledge Ziv Machnes, PhD, of Celerion for contributing to the study protocol. Medical writing assistance was provided by Erin P. Scott, PhD, of Scott Medical Communications, LLC; funding for this assistance was provided by Bryn Pharma, LLC.

### REFERENCES

1. Shaker MS, Wallace DV, Golden DBK, Oppenheimer J, Bernstein JA, Campbell RL, et al. Anaphylaxis—a 2020 practice parameter update, systematic review, and Grading of Recommendations, Assessment, Development and Evaluation (GRADE) analysis. J Allergy Clin Immunol 2020;145:1082-123.
2. Golden DBK, Wang J, Waserman S, Akin C, Campbell RL, Ellis AK, et al. Anaphylaxis: a 2023 practice parameter update. Ann Allergy Asthma Immunol 2024;132:124-76.
3. Tilley DG, Houser SR, Koch WJ. Adrenergic agonists and antagonists. In: Brunton LL, Knollmann BC, editors. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 14th ed. New York: McGraw Hill; 2023.
4. Miles LM, Ratnarajah K, Gabrielli S, Abrams EM, Protudjer JLP, Bégin P, et al. Community use of epinephrine for the treatment of anaphylaxis: a review and meta-analysis. J Allergy Clin Immunol Pract 2021;9:2321-33.
5. Gallagher M, Worth A, Cunningham-Burley S, Sheikh A. Epinephrine autoinjector use in adolescents at risk of anaphylaxis: a qualitative study in Scotland, UK. Clin Exp Allergy 2011;41:869-77.
6. Esenboga S, Ocak M, Cetinkaya PG, Sahiner UM, Soyer O, Buyuktiryaki B, et al. Physicians prescribe adrenaline autoinjectors, do parents use them when needed? Allergol Immunopathol 2020;48:3-7.
7. Turner PJ, Jerschow E, Umasunthar T, Lin R, Campbell DE, Boyle RJ. Fatal anaphylaxis: mortality rate and risk factors. J Allergy Clin Immunol Pract 2017;5:1169-78.
8. Bock SA, Muñoz-Furlong A, Sampson HA. Further fatalities caused by anaphylactic reactions to food, 2001-2006. J Allergy Clin Immunol 2007;119:1016-8.
9. Fleming JT, Clark S, Camargo CA Jr, Rudders SA. Early treatment of food-induced anaphylaxis with epinephrine is associated with a lower risk of hospitalization. J Allergy Clin Immunol Pract 2015;3:57-62.
10. Dworaczyk D, Hunt A, Lor M, Spirito MD. 13.2 mg intranasal epinephrine treatment in congestion shows increased bioavailability without pharmacokinetic and pharmacodynamic correlation. Ann Allergy Asthma Immunol 2024;133:186-193.e2.

11. Dworaczyk DA, Hunt AL, DiSpirito M, Lor M, Dretchen K, Lamson M, et al. 13.2 mg intranasal epinephrine spray demonstrates comparable pharmacokinetics, pharmacodynamics, and safety to 0.3 mg epinephrine autoinjector. J Allergy Clin Immunol Global 2023;3:100200.
12. Data on file: Bryn Pharma; 2023.
13. Dworaczyk D, Hunt A. Subanalysis of an open-label, crossover study to assess the relative bioavailability of self-administered nasal epinephrine compared to administration by trained health personnel in healthy adult subjects. J Allergy Clin Immunol 2022;149:AB2.
14. Dworaczyk D, Hunt A. 5-Period, 5-treatment crossover study to compare the pharmacokinetics of intranasal and intramuscular epinephrine administration in healthy adult participants. J Allergy Clin Immunol 2021;147:AB241.
15. Worm M, Fox AT, Wickman M, Ring J, Ebisawa M, Pouessel G, et al. Adrenaline auto injectors pharmacokinetic/pharmacodynamic studies and potential consequences for clinical practice. Clin Transl Allergy 2023;13:e12323.
16. Chooniedass R, Temple B, Martin D, Becker A. A qualitative study exploring parents' experiences with epinephrine use for their child's anaphylactic reaction. Clin Transl Allergy 2018;8:43.
17. Lieberman JA, Oppenheimer J, Hernandez-Trujillo VP, Blaiss MS. Innovations in the treatment of anaphylaxis: a review of recent data. Ann Allergy Asthma Immunol 2023;131:185-193.e10.
18. Tanimoto S, Kaliner M, Lockey RF, Ebisawa M, Koplowitz LP, Koplowitz B, et al. Pharmacokinetic and pharmacodynamic comparison of epinephrine, administered intranasally and intramuscularly: an integrated analysis. Ann Allergy Asthma Immunol 2023;130:508-514.e1.
19. Simons FE, Gu X, Simons KJ. Epinephrine absorption in adults: intramuscular versus subcutaneous injection. J Allergy Clin Immunol 2001;108:871-3.
20. Simons FE, Roberts JR, Gu X, Simons KJ. Epinephrine absorption in children with a history of anaphylaxis. J Allergy Clin Immunol 1998;101:33-7.
21. In brief. Auvi-Q–a new epinephrine auto-injector. Med Lett Drugs Ther 2013;55:13.
22. Song TT, Nelson MR, Chang JH, Engler RJ, Chowdhury BA. Adequacy of the epinephrine autoinjector needle length in delivering epinephrine to the intramuscular tissues. Ann Allergy Asthma Immunol 2005;94:539-42.
23. Kuchel GA, Avorn J, Reed MJ, Fields D. Cardiovascular responses to phlebotomy and sitting in middle-aged and elderly subjects. Arch Intern Med 1992;152:366-70.

## ONLINE REPOSITORY

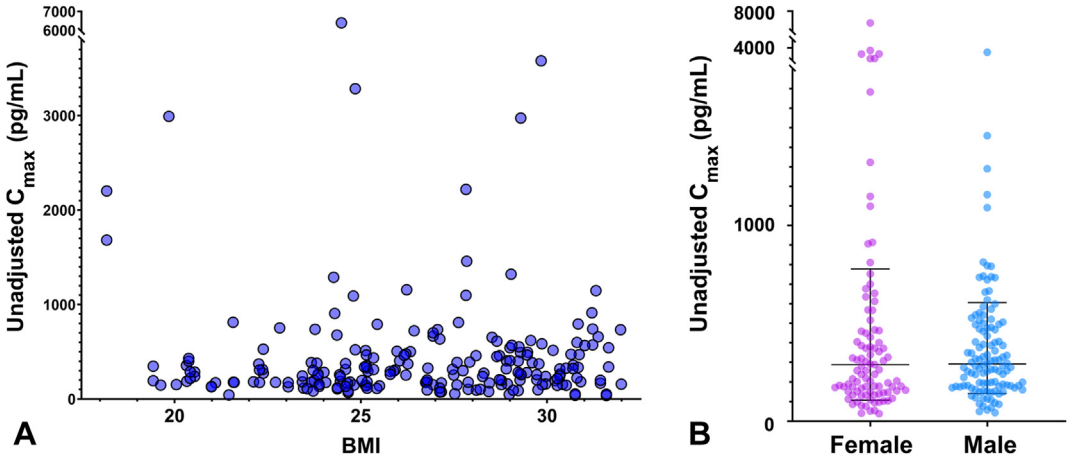


**FIGURE E1.** Effect of (**A**) BMI and (**B**) sex on 13.2 mg ENS $C_{max}$.

**TABLE E1.** Demographic characteristics of randomized participants

| Characteristic | Study 1 (n = 51) | Study 2 (n = 116) | Study 3 (n = 36) | Study 4 (n = 48) | Total (n = 251) | *P* value |
|---|---|---|---|---|---|---|
| Age (y), mean (range) | 39 (20-63) | 39 (20-65) | 40 (20-65) | 40 (20-64) | 40 (20-65) | .83 |
| Sex: male, n (%) | 26 (51) | 57 (49) | 19 (53) | 30 (63) | 132 (53) | .48 |
| Race, n (%) | | | | | | .12 |
| American Indian or Alaska Native | 1 (2) | 1 (1) | 0 | 2 (4) | 4 (2) | |
| Asian | 0 | 3 (3) | 2 (6) | 1 (2) | 6 (2) | |
| Black | 15 (29) | 26 (22) | 4 (11) | 16 (33) | 61 (24) | |
| Multiple | 4 (8) | 12 (10) | 6 (17) | 0 | 22 (9) | |
| White | 31 (61) | 74 (64) | 24 (67) | 29 (60) | 158 (63) | |
| Hispanic, n (%) | 2 (4) | 8 (7) | 3 (8) | 6 (13) | 19 (8) | .46 |
| BMI ($kg/m^2$), mean | 27.1 | 26.4 | 27.0 | 26.6 | 26.8 | .38 |
| 18.0-24.9, n (%) | 8 (16) | 39 (34) | 11 (31) | 17 (35) | 75 (30) | |
| 25.0-29.9, n (%) | 36 (71) | 64 (55) | 17 (47) | 22 (46) | 139 (55) | |
| 30.0-32.0, n (%) | 7 (14) | 13 (11) | 8 (22) | 9 (19) | 37 (15) | |
| Height (cm), mean | 171.5 | 171.7 | 170.5 | 173.0 | 171.7 | .50 |
| Weight (kg) mean | 79.8 | 77.9 | 79.4 | 79.8 | 79.2 | .75 |

n is the number of participants.