Supplementary appendix

Supplement to:

Patel N, et al. Optimal dose of adrenaline auto-injector for children and young people at risk of anaphylaxis: a phase IV randomised controlled crossover study.

CONTENTS

Supplementary Tables

1. Allocation of participants in the study
2. Additional pharmacokinetic data following injection with AAI
3. Maximum change in cardiovascular parameters in the first 20 minutes following adrenaline injection by auto-injector from baseline
4. Effect of adrenaline on cardiovascular parameters compared to baseline
5. Effect of adrenaline on stroke volume, analysed using 2-way repeated measures ANOVA
6. Comparison of AAI effect on stroke volume, using Tukey multiple comparison tests for between device differences at individual timepoints
7. Solicited adverse reactions for the first 24 hours after injection

Supplementary Figures

1. Representative ultrasound image measuring depth of injection, surface-to-bone-distance (STBD) and surface-to-muscle distance (STMD)
2. Plasma noradrenaline profile following adrenaline injection
3. Change in heart rate from baseline following adrenaline injection, by participant
4. Change in stroke volume from baseline following adrenaline injection by participant

Table S1: Allocation of participants in the study

Participant number	Visit 1		Visit 2		Emerade injected into … leg
	morning injection (am)	afternoon injection (pm)	morning injection (am)	afternoon injection (pm)	
1	Emerade 300	Epipen 300	Emerade 500	xxx	RIGHT
2	Emerade 500	xxx	Emerade 300	Epipen 300	LEFT
3	xxx	Emerade 500	Epipen 300	Emerade 300	RIGHT
4	Epipen 300	Emerade 300	xxx	Emerade 500	LEFT
5	Epipen 300	Emerade 300	xxx	Emerade 500	LEFT
6	Emerade 300	Epipen 300	Emerade 500	xxx	RIGHT
7	xxx	Emerade 500	Epipen 300	Emerade 300	RIGHT
8	Emerade 500	xxx	Emerade 300	Epipen 300	LEFT
9	Emerade 300	Epipen 300	Emerade 500	xxx	LEFT
10	Epipen 300	Emerade 300	xxx	Emerade 500	RIGHT
11	Emerade 500	xxx	Emerade 300	Epipen 300	RIGHT
12	xxx	Emerade 500	Epipen 300	Emerade 300	LEFT

Table S2: Additional pharmacokinetic data following adrenaline injection with AAI

	Emerade 300	Emerade 500	Epipen (0.3mg)
C_{max} (pg/ml)			
Arithmetic mean	269	421	345
Std Dev	145	168	246
CV	54%	40%	71%
Geometric mean	218	394	290
90% CI	(145 to 327)	(324–479)	(214–393)
C_{max1} (pg/ml)			
Arithmetic mean	216	333	326
Std Dev	112	104	255
CV	52%	31%	78%
Geometric mean	181	320	262
90% CI	(126–259)	(273–375)	(184–373)
C_{max2} (pg/ml)			
Arithmetic mean	256	393	309
Std Dev	136	180	248
CV	53%	46%	80%
Geometric mean	208	359	239
90% CI	(140–311)	(285–453)	(191–347)
T_{max1} (Time to C_{max1}) (mins)			
Arithmetic mean	15	9.6	6.3
Std Dev	18	5.0	2.3
CV	119%	52%	36%
Geometric mean	9.6	8.5	5·9
90% CI	(6.0–16)	(6.5–11)	(5.1–7.0)
T_{max2} (Time to C_{max2}) (mins)			
Arithmetic mean	58	53	35
Std Dev	29	18	20
CV	50%	33%	56%
Geometric mean	50	50	30
90% CI	(38–68)	(42–61)	(23–41)
$AUC_{0\text{-}180}$ (hr.pg/ml) (absolute values)			
Arithmetic mean	428	609	391
Std Dev	175	231	110
CV	41%	38%	28%
Geometric mean	380	576	377
90% CI	(281–514)	(483–686)	(325–437)
$AUC_{0\text{-}180}$ (hr.pg/ml) (baseline corrected)			
Arithmetic mean	255	455	233
Std Dev	167	271	123
CV	66%	59%	53%
Geometric mean	174	387	203
90% CI	(98–311)	(283–531)	(152–273)
$AUC_{0\text{-}60}$ (hr.pg/ml) (baseline corrected)			
Arithmetic mean	112	218	132
Std Dev	71	117	82
CV	63%	54%	62%
Geometric mean	75	188	109
90% CI	(41–138)	(138-255)	(76–155)
$AUC_{0\text{-}30}$ (hr.pg/ml) (baseline corrected)			
Arithmetic mean	48	92	78
Std Dev	35	44	61
CV	73	48	78
Geometric mean	33	82	56
90% CI	(18–58)	(62–108)	(34–92)

Table S3: Maximum change in cardiovascular parameters in the first 20 minutes following adrenaline injection by auto-injector from baseline. Mean change (SD).

	Epipen 300µg	**Emerade 300 300µg**	**Emerade 500 500µg**
Heart rate (bpm)	14.5 (8.3) Mean increase 20%	8.5 (6.5) Mean increase 11%	9.2 (4.4) Mean increase 13%
Stroke volume (ml/m^2)	1.6 (9.1) Mean increase 3%	8.8 (11.5) Mean increase 18%	13.1 (13.0) Mean increase 30%
Cardiac output (l/min/m2)	0.5 (0.7) Mean increase 14%	0.8 (1.0) Mean increase 21%	1.2 (1.0) Mean increase 40%
sBP (mmHg)	11.2 (11.6) Mean increase 11%	14.8 (13.7) Mean increase 15%	13.5 (7.6) Mean increase 13%
dBP (mmHg)	6.9 (12.2) Mean increase 12%	8.6 (16.9) Mean increase 14%	6.2 (14.7) Mean increase 10%

Table S4:

Effect of adrenaline on cardiovascular parameters compared to baseline, by one-way ANOVA

A one-way ANOVA was performed to compare the effect of ***time*** post injection on each CVS parameter in turn. Where the overall assessment demonstrated significance, a further analysis was undertaken comparing each post-injection timepoint to baseline.

Heart Rate

HR	**ANOVA summary**	**Mean difference from baseline** (adj.p-value)					
		5min	10min	15min	20min	25min	30min
Epipen 0.3mg	F(2,21.7) = 6.4 **p=0.0066**	**12.4 (0.0026)**	**7.2 (0.0070)**	4.7 (0.051)	4.1 (0.076)	**4.4 (0.036)**	4.2 (0.21)
Emerade 300µg	F(2.8,30.5) = 3.3 **p=0.037**	6.1 (0.084)	3.7 (0.14)	2.7 (0.070)	1.7 (0.39)	2.4 (0.28)	2.8 (0.18)
Emerade 500µg	F(3.5, 38.7) = 4.4 **p=0.0064**	**5.9 (0.0019)**	**4.9 (0.022)**	**6.2 (0.017)**	**4.0 (0.0052)**	**4.5 (0.0022)**	**4.7 (0.010)**

Stroke Volume

SV	**ANOVA summary**	**Mean difference from baseline** (adj.p-value)					
		5min	10min	15min	20min	25min	30min
Epipen 0.3mg	F(2.9, 30.9) =0.84 p=0.48	-	-	-	-	-	-
Emerade 300µg	F(3.2,35.6) = 1.6 p=0.20	-	-	-	-	-	-
Emerade 500µg	F(2.4, 25.9) = 4.4 **p=0.018**	8.4 (0.27)	9.4 (0.13)	11.1 (0.06)	11.0 (0.05)	9.0 (0.18)	10.3 (0.062)

Cardiac Output

CO	**ANOVA summary**	**Mean difference from baseline** (adj.p-value)					
		5min	10min	15min	20min	25min	30min
Epipen 0.3mg	F(2.9, 32.0) =0.60 p=0.62	-	-	-	-	-	-
Emerade 300µg	F(2.6,28.8) = 2.5 p= 0.084	-	-	-	-	-	-
Emerade 500µg	F(2.1, 23.4) = 7.0 **p=0.0037**	0.38 (0.069)	0.41 (0.11)	**0.60 (0.011)**	**0.43 (0.024)**	**0.41 (0.043)**	0.44 (0.29)

Systolic BP

sBP	**ANOVA summary**	**Mean difference from baseline** (adj.p-value)					
		5min	10min	15min	20min	25min	30min
Epipen 0.3mg	F (6, 24) =6.3 **p=0.0005**	**21.9 (<0.0001)**	6.1 (0.38)	3.9 (0.76)	1.5 (>0.99)	5.7 (0.53)	6.8 (0.21)
Emerade 300µg	F(6, 27) = 2.6 **p= 0.040**	11.1 (0.24)	5.6 (0.95)	14.9 (0.073)	-2.3 (>0.99)	-6.7 (0.80)	4.4 (0.97)
Emerade 500µg	F(6, 27) = 4.5 **p=0.0027**	**15.7 (0.0057)**	3.4 (0.90)	**18.7 (0.0009)**	7.0 (0.30)	5.0 (0.82)	6.8 (0.43)

Diastolic BP

dBP	**ANOVA summary**	**Mean difference from baseline** (adj.p-value)					
		5min	10min	15min	20min	25min	30min
Epipen 0.3mg	F(6, 24) = 2.6 **p=0.045**	**15.4 (0.01)**	-2.9 (0.96)	6.1 (0.47)	1.2 (>0.99)	3.9 (0.92)	0.6 (>0.99)
Emerade 300µg	F(6,27) = 0.74 p= 0.63	-	-	-	-	-	-
Emerade 500µg	F(6, 27) = 1.2 p=0.36	-	-	-	-	-	-

Table S5: 2-way repeated measures ANOVA analysing the effect of "AAI" and "time post-injection" on the change in stroke volume from baseline stroke volume.

	% of total variation	F value	P value
Time x Device Interaction	0.4	0.44	0.948
Time effect	1.4	2.86	**0.048**
Device effect	18.2	4.73	**0.016**
Subject (participant) effect	63.6	23.3	**<0.0001**

Table S6: Comparison of AAI effect on stroke volume, using Tukey multiple comparison tests for between device differences at individual timepoints

Timepoint following adrenaline injection	Epipen (300µg) vs Emerade 300 (300µg)	Epipen (300µg) vs Emerade 500 (500µg)	Emerade 300 (300µg) vs Emerade 500 (500µg)
5 min	-7.6 (-16.7 to 1.5) p=0.11	**-13.0 (-25.7 to -0.22) p=0.046**	-5.4 (-18.0 to 7.3) p=0.53
10 min	-7.7 (-18.7 to 3.3) p=0.21	-11.5 (23.4 to 0.35) p=0.058	-3.8 (-16.7 to 9.1) p=0.74
15 min	-7.8 (-17.5 to -1.8) p=0.13	**-14.3 (-26.3 to -2.3) p=0.018**	-6.5 (-18.4 to 5.5) p=0.38
20 min	-7.0 (-17.5 to 3.6) p=0.24	**-13.4 (-25.6 to -1.2) p=0.030**	-6.4 (-17.9 to 5.1) p=0.35
30 min	-4.5 (-15.7 to 6.6) p=0.57	**-12.9 (-25.4 to -0.3) p=0.044**	-8.3 (-19.6 to 3.0) p=0.18
45 min	-4.0 (-15.4 to 7.5) p=0.66	-10.6 (-24.3 to 3.2) p=0.15	-6.6 (-18.6 to 5.4) p=0.36
60 min	-8.3 (-18.3 to 1.7) p=0.11	**-13.1 (-25.8 to -0.30) p=0.044**	-4.7 (-16.1 to 6.6) p=0.54

Data presented as mean difference in Stroke Volume (SV) between the first device in the comparison compared to the compator device, together with 95%CI of the difference. For example, the mean difference in SV at 5 minutes following Epipen 0.3mg injection was 7.6 ml/m^2 lower compared to the change in SV after injection with Emerade 300µg at the same timepoint.

P values are adjusted for multiple comparisons.

Table S7: Solicited adverse reactions for the first 24 hours after injection.

	Emerade 300 (n=12)	Emerade 500 (n=12)	Epipen (0·3mg) (n=12)
Local			
Pain at injection site (minor)	8 (67%)	6 (50%)	7 (58%)
Bleeding at injection site (minor)	1 (8%)	1 (8%)	3 (25%)
Blanching at injection site	0 (0%)	1 (8%)	1 (8%)
Systemic			
Overall	4 (33%)	9 (75%)	8 (67%)
Cardiac	3 (25%)	4 (33%)	3 (25%)
• Palpitations (mild, transient)			
Gastrointestinal	0 (0%)	0 (0%)	0 (0%)
Nervous system			
• Tremor	3 (25%)	7 (58%)	8 (67%)
• Headache	1 (8%)	0 (0%)	1 (8%)
• Dizziness	1 (8%)	1 (8%)	0 (0%)
AEs reported after 24 hours			
Bruising at injection site	1 (8%)	0 (0%)	1 (8%)
Muscle ache at injection site	2 (17%)	0 (0%)	1 (8%)

Figure S1: Representative ultrasound image measuring depth of injection, surface-to-bone-distance and surface-to-muscle distance in participants.

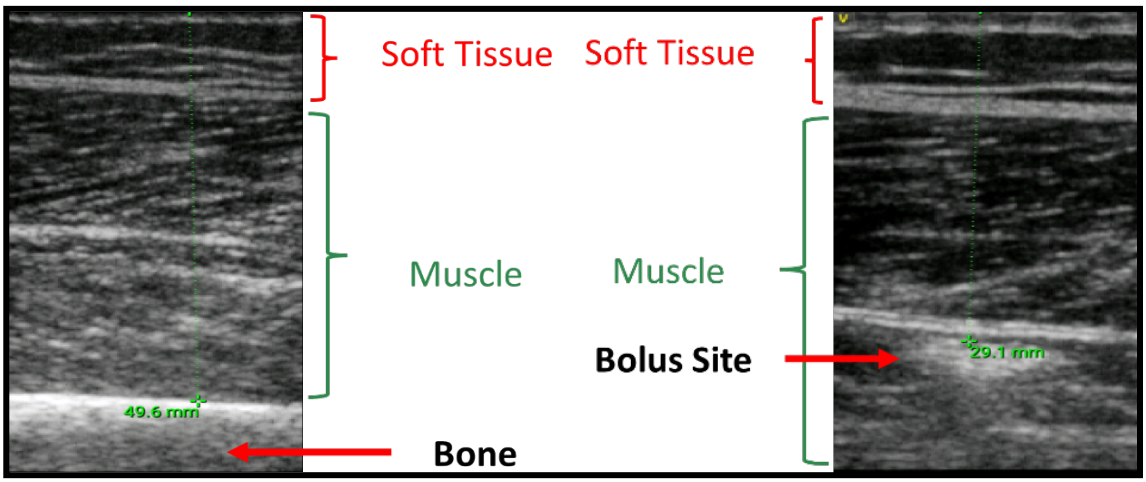

Figure S2: Plasma noradrenaline profile following adrenaline injection with 3 different devices. Data are arithmetic mean±SEM (n=12).

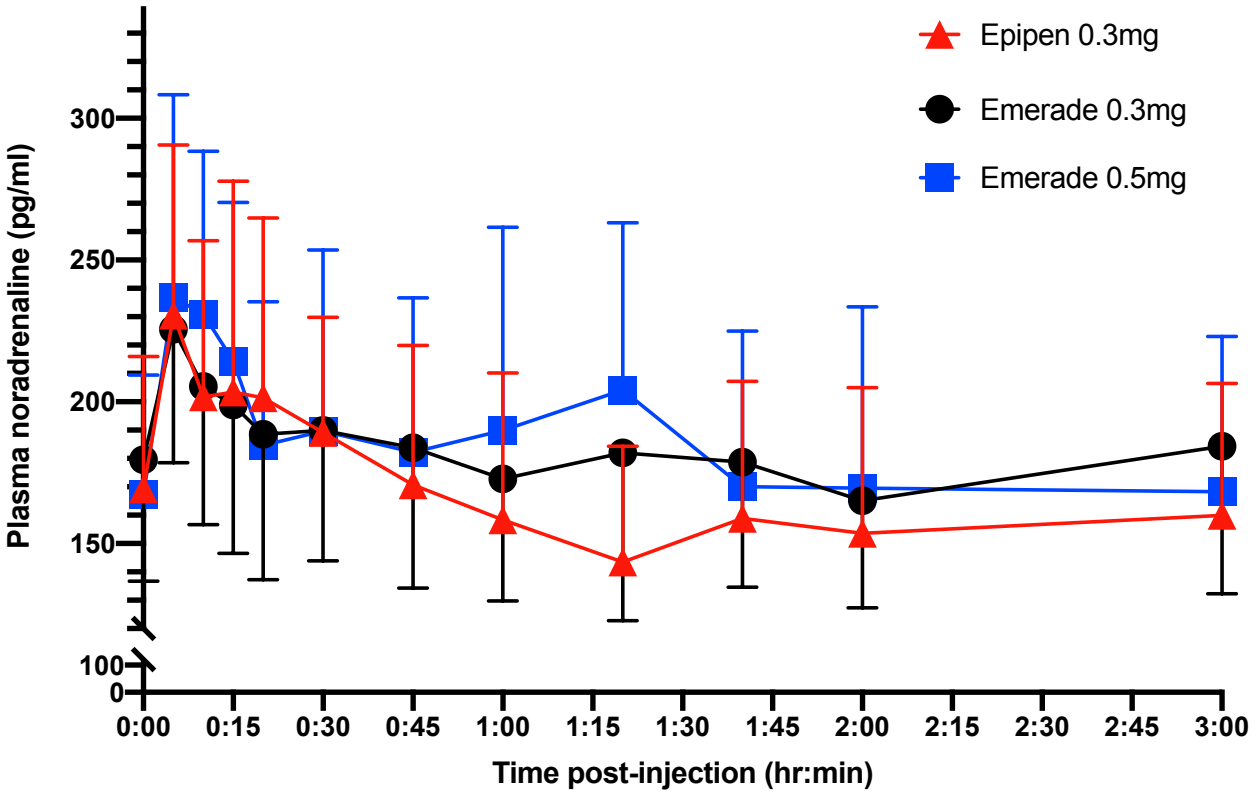

Figure S3: Change in heart rate from baseline following adrenaline injection with 3 different devices, by participant (n=12).

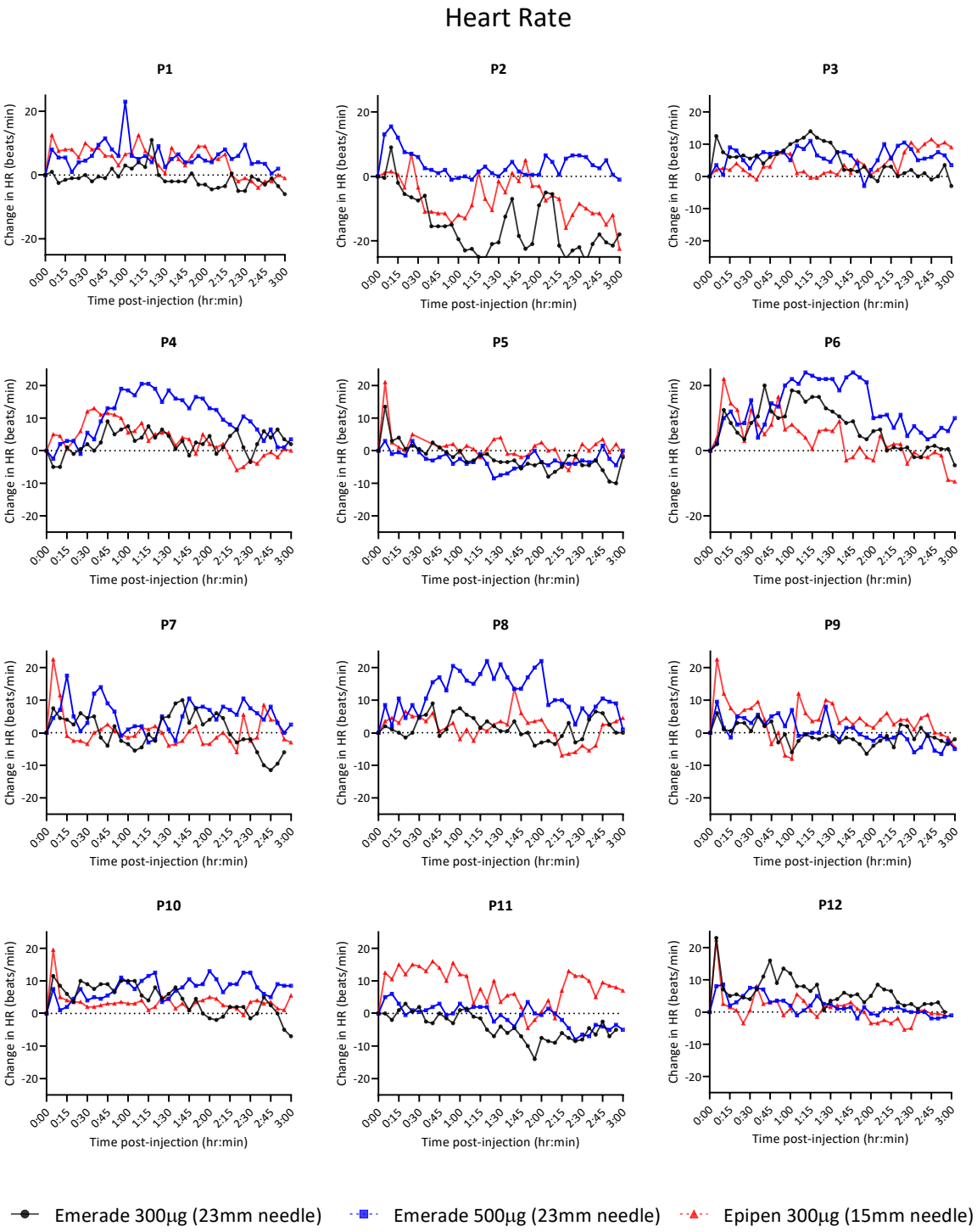

Figure S4: Change in stroke volume from baseline following adrenaline injection with 3 different devices, by participant (n=12).

