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Impact of Personal Protective Equipment on Pediatric Cardiopulmonary Resuscitation Performance: A Controlled Trial

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Abstract

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Objectives—To determine whether personal protective equipment (PPE) results in deterioration in chest compression (CC) quality and greater fatigue for administering health care providers (HCPs).

Methods—In this multicenter study, HCPs completed 2 sessions. In session 1 (baseline), HCPs wore normal attire; in session 2, HCPs donned full PPE. During each session, they performed 5 minutes of uninterrupted CCs on a child manikin. CC rate, depth, and release velocity were reported in 10 30-second epochs. Change in CC parameters and self-reported fatigue were measured between the start and 2- and 5-minute epochs.

Results—We enrolled 108 HCPs (prehospital and in-hospital providers). The median CC rate did not change significantly between epochs 1 and 10 during baseline sessions. Median CC depth and release velocity decreased over 5 minutes with PPE. There were no significant differences in CC parameters between baseline and PPE sessions in any provider group. Median fatigue scores during baseline sessions were 2 (at start), 4 (at 2 minutes), and 6 (at 5 minutes). There was a significantly higher median fatigue score between 0 and 5 minutes in both study sessions and in all groups. Fatigue scores were significantly higher for providers wearing PPE compared with baseline specifically among prehospital providers.

Conclusions—During a clinically appropriate 2-minute period, neither CC quality nor self-reported fatigue worsened to a significant degree in providers wearing PPE. Our data suggest that Pediatric Basic Life Support recommendations for CC providers to switch every 2 minutes need not be altered with PPE use.

High-quality cardiopulmonary resuscitation (CPR) remains the cornerstone of therapy for cardiac arrest. According to guidelines by the American Heart Association (AHA) for optimizing CPR quality, it is recommended that a single provider perform CPR for no longer than 2 minutes before switching with another provider.[1] This recommendation is based on published literature showing that fatigue leads to the deterioration of chest compression (CC) quality within minutes.[2] Published data on simulated pediatric CPR have demonstrated that delivering high-quality CC is associated with a degree of work comparable to high-intensity aerobic exercise.[3] Following the 1995 Tokyo subway sarin attack, and more recently the 2015 Ebola viral disease outbreak, hospitals in the United States have focused on health care provider (HCP) preparedness to use personal protective equipment (PPE) during clinical care. PPE is a term used to refer to barrier clothing, gloves, and/or headgear designed to protect an individual from harmful exposure. Within the health care context, these exposures are typically from infectious or toxic materials. Little is known about the impact of PPE on physical fatigue during clinical care, including CPR. There have been several studies examining the impact of wearing PPE during simulated resuscitative clinical tasks in adult models, including CPR, where PPE use has been shown to result in poorer CC quality and increased fatigue in providers.[4] In particular, no published study to date has evaluated the impact of PPE on CPR in a pediatric model.

For the present study, we sought to examine the effect of PPE on HCPs' ability to maintain high-quality CCs in a simulated pediatric patient. These data were obtained in the setting of a larger trial designed to assess the impact of PPE use on common resuscitative procedures in simulated pediatric resuscitation. We hypothesized that CC quality would deteriorate

faster and provider fatigue would increase to a greater extent in providers administering CPR with PPE compared with normal attire.

Methods

Participants

This was a prospective, multisite simulation study at 3 tertiary pediatric centers. The study was approved by the local institutional review boards at the three tertiary care centers, and participants provided verbal consent. Data on CPR performance were collected for a planned prospective analysis in the context of a larger trial of pediatric procedural skills while wearing PPE.[5] Eligible participants were HCPs (nurses, physicians, and paramedics) who (a) would perform the procedures to be studied as part of their job responsibilities, (b) had received their institution's PPE training, (c) were in their role for at least 1 year, and (d) had no contraindication to wearing PPE. Providers who were not likely to have sufficient procedural experience (e.g., first-year fellows in emergency medicine or critical care medicine), providers whose scope of practice did not include CPR, and providers with physical limitations to performing a prolonged period of simulated CPR were excluded.

Each participant attended 2 study sessions. For the first session (baseline), participants wore normal attire. For the second session, participants wore the institutionally approved full-body PPE. For prehospital providers, this consisted of a Level B, encapsulating, non-gas tight suit with a self-contained breathing apparatus; for hospital providers, this consisted of a full-body suit with a personal air purifying respirator (Level C). Each study site used their own PPE equipment, all of which was chosen by their respective institutions based on Occupational Safety and Health Administration and Environmental Protection Agency standards and classification systems. Participants were required to perform 5 minutes of uninterrupted CCs on a pediatric manikin simulating a 5-year-old child (MegaCode Kid, Laerdal Medical, Stavanger, Norway) placed on a stretcher located 28 inches above the floor. Participants were instructed to stop CCs at any time if they felt too fatigued to continue. The 2 study sessions were scheduled a minimum of 2 weeks apart to minimize the possibility of bias from fatigue following the first session.

Measures

A device using pressure sensor/accelerometer/impedance technology to measure CC quality was applied to the manikin during all study sessions (R Series, Zoll Medical, Chelmsford, MA, USA). This device measures CC parameters and summarizes average values over user-specified time periods. For the present study, CPR parameters were measured in 30-second epochs, with each participant completing a maximum of 10 consecutive epochs per study session. While the device is made to be used as a source of real-time visual and audio feedback to a participant performing CCs, for the present study the feedback features were not made available to the participant (audio was silenced and the feedback display was facing away from provider) so as not to influence CC quality. This methodology of measuring CC quality has been reported in other simulation studies.[6–8]

CC parameters measured for the current study were rate, depth, and release velocity (RV). Current Pediatric Advanced Life Support guidelines for a 5-year-old child would recommend a compression rate of 100–120 per minute and a depth of 1/3 to 1/2; of the anteroposterior diameter of the chest, corresponding to a depth of at least 2 inches but not more than 2.5 inches.[1] RV is a measure of the speed of chest wall recoil (in mm/second); higher RV has been associated with improved outcomes from out-of-hospital cardiac arrest in adults.[9] No published data on RV in pediatric CPR currently exist.

At 60-second increments, participants were prompted by the facilitator to self-report their level of fatigue on a 10-point scale (0=no fatigue, 10=extreme fatigue). These scores were compared to a fatigue score obtained at the beginning of the CPR period.

Analysis

Due to the difference in type of PPE used between the two provider groups, data were analyzed separately for prehospital and hospital providers. All data were summarized descriptively with frequencies and percentages for categorical data and medians and interquartile ranges (IQRs) for continuous data. The changes in each CC parameter and in self-reported fatigue were calculated between 2 pairs of time points: time 0 and at 5 minutes (the entire CC period), and time 0 and at 2 minutes (the recommended duration of a single chest compression provider, per AHA guidelines). Medians for CPR parameters were compared during 30-second epochs (i.e., the first 30 seconds of compressions and the final 30 seconds within the time period of interest). Medians for fatigue scores were compared as reported at the beginning of the CPR period and at the end of the 1-minute period of interest (either at 2 minutes or at 5 minutes). The median changes were tested using the Wilcoxon signed-rank test. The mean change of each CC parameter during baseline and PPE sessions for both provider groups was compared using mixed models to account for correlations due to multiple observations within a participant. Session (baseline and PPE), time, and session-by-time interaction were included as fixed effects, and participant was included as a random effect. Session-by-time interaction was tested to examine whether there were significant differences in mean trajectories between baseline and PPE sessions. Mixed models were also used to compare the mean trajectories of fatigue score between the 2 study sessions for both provider groups and to examine the mean increases in fatigue scores from baseline between 2 provider groups. All tests were 2-sided; $p<0.05$ was considered to be statistically significant. Statistical analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

A total of 108 participants completed both study sessions (prehospital n=48; nurse n=30; physician n=30) and were included in the analysis. Median (range) age for the 108 participants in the analysis was 40 (24–60) years. The majority of the participants were male (52.8%), white (75.0%), and not of Hispanic or Latino ethnicity (85.2%). The median time between baseline and PPE sessions was 5.7 weeks, and 58.3% of participants had previously worn PPE >10 times in their career (Table 1).

Altogether, 106/108 participants (98%) completed 5 minutes of CCs without stopping during both sessions. Medians and ranges of CPR parameters across all participants during baseline and PPE sessions stratified by 30-second epochs are shown in Table 2. CPR parameters (rate, depth, RV) during each study session stratified by provider group (prehospital vs. hospital [nurses and physicians]) are displayed in Figure 1. There were no statistically significant differences in CC parameters between baseline and PPE sessions in any provider group.

The differences in specific CPR parameters when measured over 2 minutes and over 5 minutes are shown in Table 3. RV was significantly slower at either times 2 or 5 minutes when compared with time 0 in all provider groups (p for all <.001). Chest compression depth decreased significantly at either time 2 or 5 minutes during the PPE session in all provider groups (p for all <.001); among hospital providers, CC depth decreased with time significantly in the baseline session, but no difference was found for prehospital providers. The CC rate did not change over 5 minutes in either session among prehospital providers. In contrast, the CC rate increased significantly over 5 minutes in the PPE session among hospital providers (median change=3 cpm, p=0.04).

Self-reported fatigue scores by 1-minute increments are shown in Figure 2. Increase in fatigue was similar between hospital personnel and prehospital personnel (baseline: p=0.26; PPE: p=0.13). Prehospital providers reported a higher level of fatigue over the 5-minute study session in PPE compared with baseline (p=0.02). Increase in fatigue scores was similar between baseline and PPE sessions among hospital providers (p=0.82). The difference in reported fatigue between time 0 and either 2 or 5 minutes was significant in all provider groups (Table 4; p for both <.001).

Discussion

In this study of experienced clinicians performing simulated pediatric CCs, we found that CC quality during CPR did not differ significantly between baseline and PPE sessions. This finding was contrary to our hypothesis and suggests that existing AHA guidelines of switching chest compression providers every 2 minutes during pediatric CPR need not be altered in the setting of PPE use. Self-reported fatigue during CPR was significantly higher at 2 and 5 minutes than at baseline; the increase in fatigue scores was significantly greater in the prehospital group wearing PPE compared to baseline, but this difference was not significant in hospital providers.

A secondary finding of our study was that experienced providers, both in and out of PPE, failed to exhibit any significant deterioration in CC rate or depth over a 5-minute period. Self-reported fatigue increased for the majority of participants between 0 and 5 minutes, but this subjective fatigue was not commensurate with any measurable change in CPR rate or depth. Additionally, RV was shown to significantly decrease over time, suggesting that providers were applying a greater degree of residual leaning force as time went on.

Chest compression quality has been shown to deteriorate within minutes for individual providers in manikin and human studies.[2,3] It is assumed that this deterioration is due at

least in part to physical fatigue. Badaki-Makun et al. published a manikin study demonstrating that work output during CC performance is comparable to that of running at 9 km/hour or swimming at high intensity.[3] Current AHA guidelines continue to recommend switching compressors every 2 minutes to minimize the influence of fatigue on CC quality.[1] However, even transient interruptions in continuously applied chest compressions has been shown to result in a loss of accumulated coronary perfusion pressure, potentially impacting the likelihood of return of spontaneous circulation from cardiac arrest.[10] Additionally, switching compressors more often than is necessary may be associated with an increase in interruptions and a decreased CC fraction. Sutton et al. reported on the impact of pauses in CPR on compression quality, finding that CCs following a provider switch had shallower depth and greater leaning force.[11] Donoghue et al. reported on a videographic analysis of CPR on actual patients in a pediatric resuscitation bay and found that individual providers performed CCs for <120 seconds in 76% of compression segments, but also that 40% of CC segments were <60 seconds.[12] Data from the present study may suggest that, for some experienced providers, continuing CCs beyond the recommended 2 minutes of duration may be possible without any significant change in CC quality, which in turn may minimize the interruptions and alterations in CC quality associated with changes in compressors. This may be particularly important in a situation where PPE is clinically indicated (i.e., toxic or infectious exposure), given the logistic burden of having multiple providers in PPE available for a given patient requiring CPR. In other words, there is an intuitive advantage to determining the minimal number of providers who would need to be present in PPE and able to maintain high-quality CPR for longer periods, while at the same time ensuring provider safety from the fatigue of CPR.

The use of PPE during CPR has been studied in adult manikins. Chen et al. reported on anesthesia residents performing CCs on an adult manikin in and out of PPE, and found that compression rate and depth were worse when wearing PPE.[4] The group additionally reported that providers reported greater fatigue and exhibited a greater increase in their measured heart rate after 2 minutes of CCs while wearing PPE compared with normal attire. Methodological differences between that study and the present study include the use of an adult manikin lying on the ground as opposed to a stretcher, and having participants perform only 2 minutes of CCs. Median compression depth in their study ranged from 1.7–1.9 inches, which is not compliant with the recommended depth of 2–2.5 inches. Reasons for these differences are unclear, although it could be inferred that their participants (anesthesia residents) have less experience with performing CPR than the experienced participants in our study.

An important consideration for the present study is that applying the results to a realistic clinical setting may be challenging. Specifically, field triage protocols for mass casualty or disaster events where PPE would be indicated often dictate that victims who are in cardiac arrest do not receive CPR and are declared ‘dead’ by prehospital personnel.[13] For in-hospital providers caring for patients with a communicable disease such as Ebola, it may be reasonable to assume that in-hospital cardiac arrest in such patients due to refractory shock and/or respiratory insufficiency is very unlikely to respond to resuscitative efforts and CPR could plausibly be regarded as a futile intervention. Nonetheless, for providers wearing PPE in settings where some degree of survivability of a patient with cardiac arrest might be

present (e.g., an Ebola patient with cardiac arrest due to sudden dysrhythmia, patients in decontamination areas prior to hospital entry), our data should serve as assurance that effective CPR can still be achieved by experienced providers wearing PPE.

Several limitations of the present study should be mentioned. As with all manikin-based studies, it is possible that study participants may have performed tasks in a manner that does not truly reflect the way they would perform the analogous tasks in a real clinical situation. For the present study we believe that this biasing influence is minimal for 2 reasons. Firstly, all of our participants are experienced clinicians in centers and clinical areas where simulation education is a routine part of ongoing educational programs, and all participants were familiar with the concept of 'suspension of disbelief' applied to simulation-based learning. Secondly, the study session in question consisted of a single psychomotor skill, with no clinical or cognitive context applied and no requirement of decision making or inference on the part of participants. It is important, however, to mention that our study does not take into account certain important elements of clinical care with PPE such as time to don the equipment, the interference of the equipment with communication, and difficulty with performing physical assessments.

Each participant was asked to perform 5 minutes of uninterrupted CCs; this duration of CPR is contrary to what is normally recommended and did not include ventilations, as would normally be recommended during CPR by trained HCPs. These experimental conditions were deliberately chosen based on our hypothesized assumption that the onset of fatigue, as evidenced by either self-report or measurable deterioration in CC quality, would be determined in a greater number of participants if the duration of the study session was longer. Our findings did not support this hypothesis. Additionally, it may not be possible to extrapolate our findings to a clinical setting where CPR is being performed by a full team of providers with the appropriate inclusion of ventilation, pauses for reassessment, defibrillation, and other elements of CPR.

Our assessment of fatigue for the present study was based on self-report, which may be prone to bias depending on the providers' perspective. We attempted to control for bias by measuring reported fatigue at baseline as well as consistent assurance during study session orientation that no qualitative performance assessment was being conducted; nonetheless, we believe that underreporting of fatigue may still have been possible. Published literature has used more sophisticated techniques, such as vital sign monitoring, to measure changes in participant heart rate and/or temperature, or an exercise physiology laboratory to quantify oxygen consumption during simulated CPR.[3,4] These techniques were beyond the scope of our methodology but may be considered in future studies of the impact of PPE on clinical performance. Additionally, as mentioned above, it may not be possible to extrapolate the self-reported fatigue data measured in this simulated setting to the degree of fatigue a provider would experience during care of an actual child in cardiac arrest; it is likely that a specific clinical context (leading to interruptions, real-time adjustments of CC technique, etc.) and/or the heightened degree of anxiety associated with a critical event such as cardiac arrest might lead to differences in fatigue.

Conclusions

In a simulated pediatric cardiac arrest patient, the use of PPE did not result in significantly worsened CC quality or self-reported fatigue in experienced HCPs. Further research in this area should examine whether performing CPR in a more realistic dynamic clinical scenario yields different results, and whether objective measurement of provider physiology more reliably determines degree of fatigue during CPR in PPE.

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Best Pharmaceuticals for Children Act – Pediatric Trials Network

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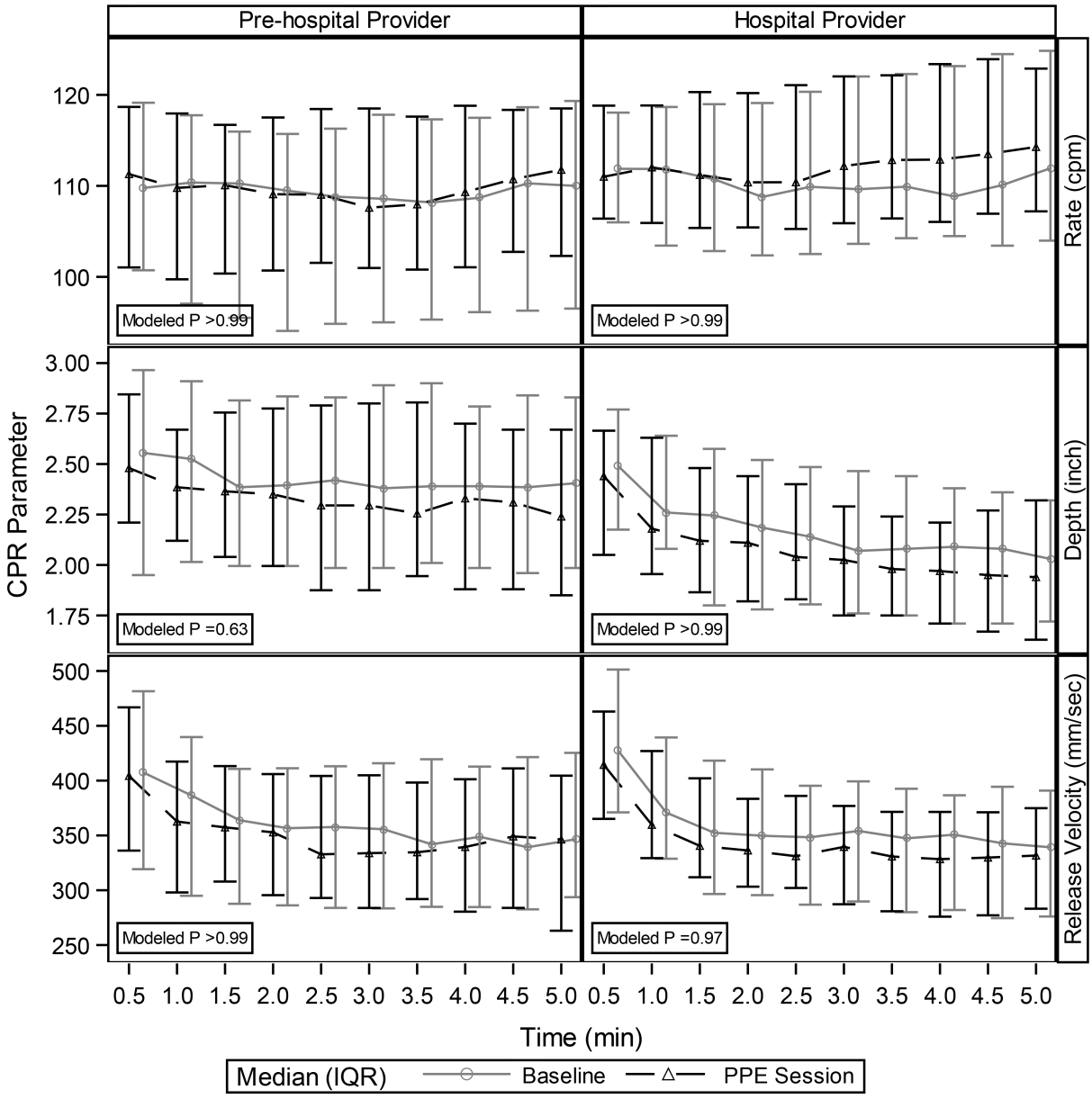

Figure 1.
CPR parameters during baseline and PPE sessions stratified by provider group

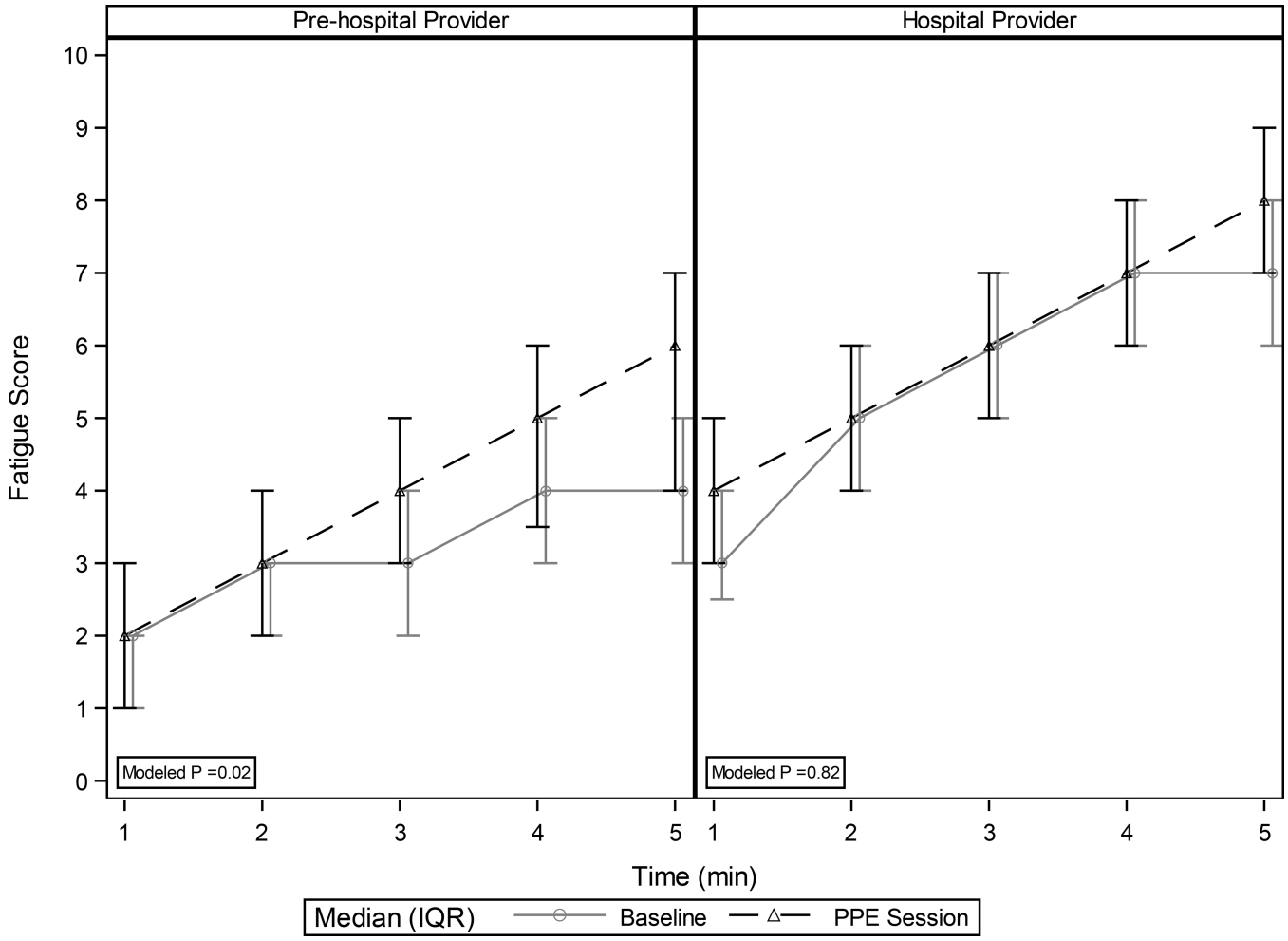

Figure 2.
Fatigue scores during baseline and PPE sessions stratified by provider group

Table 1.

Demographic and Baseline Characteristics

	Prehospital Provider (N=48)	Hospital Provider		Total (N=108)
		Physician (N=30)	Nurse (N=30)	
Age, years				
Median (IQR)	38.5 (33.5–44.5)	44.5 (38.0–51.0)	36.5 (30.0–46.0)	40.0 (34.0–47.0)
Range	28.0–54.0	31.0–58.0	24.0–60.0	24.0–60.0
Sex, n (%)				
Male	45 (93.8%)	11 (36.7%)	1 (3.3%)	57 (52.8%)
Ethnicity, n (%)				
Hispanic or Latino	5 (10.4%)	1 (3.3%)	3 (10.0%)	9 (8.3%)
Not Hispanic or Latino	39 (81.3%)	26 (86.7%)	27 (90.0%)	92 (85.2%)
Ethnicity not reported	4 (8.3%)	3 (10.0%)	0	7 (6.5%)
Race, n (%)				
White	34 (70.8%)	22 (73.3%)	25 (83.3%)	81 (75.0%)
Black or African American	5 (10.4%)	1 (3.3%)	0	6 (5.6%)
Asian	1 (2.1%)	5 (16.7%)	2 (6.7%)	8 (7.4%)
More than one race	5 (10.4%)	2 (6.7%)	3 (10.0%)	10 (9.3%)
Race not reported	3 (6.3%)	0	0	3 (2.8%)
Weeks between baseline and PPE sessions				
Median (IQR)	16.0 (4.9–16.4)	5.7 (3.4–9.0)	4.7 (2.9–6.0)	5.7 (4.4–16.0)
Range	3.4–24.4	2.0–52.0	1.1–28.0	1.1–52.0
Number of times participant has worn PPE in career, n (%)				
1 – 2	1 (2.1%)	9 (30.0%)	2 (6.7%)	12 (11.1%)
3 – 5	2 (4.2%)	11 (36.7%)	6 (20.0%)	19 (17.6%)
6 – 10	3 (6.3%)	6 (20.0%)	5 (16.7%)	14 (13.0%)
>10	42 (87.5%)	4 (13.3%)	17 (56.7%)	63 (58.3%)

PPE = personal protective equipment; IQR = interquartile range.

Table 2.

CPR Parameters Over 5 Minutes During Baseline and PPE Sessions

	Time (minute)									
	0.5	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**
Chest Compression Rate, cpm										
Baseline										
N	108	108	108	108	108	108	107	107	107	106
Median (IQR)	111 (103–118)	111 (100–118)	110 (99–118)	109 (99–118)	109 (99–118)	109 (98–119)	109 (100–121)	109 (100–120)	110 (102–121)	111 (102–123)
Range	51–158	43–160	42–157	43–157	40–155	36–149	39–146	37–148	38–146	39–147
PPE										
N	108	108	108	108	108	108	107	107	107	106
Median (IQR)	111 (104–119)	112 (104–118)	111 (103–119)	110 (103–119)	110 (103–120)	110 (103–120)	112 (104–120)	112 (104–121)	113 (104–121)	113 (104–122)
Range	54–142	47–138	47–139	47–141	47–143	47–145	49–144	48–146	47–148	49–152
Chest Compression Depth, inches										
Baseline										
N	108	108	108	108	108	108	107	107	107	106
Median (IQR)	2.5 (2.1–2.8)	2.4 (2.1–2.8)	2.3 (1.9–2.7)	2.2 (1.9–2.7)	2.2 (1.9–2.7)	2.2 (1.9–2.6)	2.2 (1.9–2.6)	2.2 (1.9–2.6)	2.2 (1.8–2.6)	2.1 (1.8–2.5)
Range	1.3–4.1	1.3–4.2	1.2–4.2	1.1–4.3	1.1–4.2	0.9–4.0	0.9–4.1	0.9–4.3	1.0–4.3	0.9–4.3
PPE										
N	108	108	108	108	108	108	107	107	107	106
Median (IQR)	2.5 (2.1–2.8)	2.4 (2.0–2.7)	2.2 (1.9–2.6)	2.2 (1.9–2.6)	2.1 (1.8–2.5)	2.1 (1.8–2.5)	2.1 (1.8–2.5)	2.1 (1.7–2.5)	2.1 (1.7–2.4)	2.1 (1.7–2.5)
Range	1.2–4.0	1.1–3.9	1.1–3.6	1.1–3.9	1.0–4.0	1.1–4.1	1.1–4.2	1.1–4.3	1.0–4.3	1.1–4.2
Release Velocity, mm/s										
Baseline										
N	108	108	108	108	108	108	107	107	107	106
Median (IQR)	418 (335–492)	371 (306–439)	357 (288–413)	351 (293–411)	356 (287–409)	354 (286–404)	346 (284–397)	350 (284–398)	342 (279–400)	343 (286–397)
Range	185–786	169–678	167–613	172–624	172–606	165–715	167–554	164–559	172–560	167–580
PPE										
N	108	108	108	108	108	108	107	107	107	106
Median (IQR)	414 (355–467)	360 (318–424)	343 (310–406)	340 (301–400)	333 (299–395)	339 (286–387)	333 (283–390)	335 (276–392)	340 (279–389)	339 (282–392)
Range	140–710	137–590	145–538	145–540	134–546	144–558	145–546	140–561	137–558	131–578

CPR = cardiopulmonary resuscitation; PPE = personal protective equipment; IQR = interquartile range; cpm = compressions per minute.

Table 3.

Change in CPR Parameters Over 2 and 5 Minutes by Provider Group

		Prehospital Provider						Hospital Provider					
		Baseline			PPE			Baseline			PPE		
CPR Parameter	**Time Points Compared**	**N**	**Median (IQR)**	**P-value**	**N**	**Median (IQR)**	**P-value**	**N**	**Median (IQR)**	**P-value**	**N**	**Median (IQR)**	**P-value**
Chest Compression Rate, cpm	Time 0 to 2 Minutes	48	−3 (−8–1)	<.001	48	−2 (−5–1)	0.0089	60	−2 (−5–1)	0.0027	60	−1 (−5–2)	0.06
	Time 0 to 5 Minutes	48	−2 (−7–4)	0.26	47	−1 (−5–7)	0.9502	58	1 (−4–5)	0.5764	59	3 (−3–6)	0.04
Chest Compression Depth, inches	Time 0 to 2 Minutes	48	−0.1 (−0.3–0.2)	0.13	48	−0.1 (−0.3–0.0)	<.0001	60	−0.3 (−0.5–−0.1)	<.0001	60	−0.3 (−0.5–−0.1)	<.001
	Time 0 to 5 Minutes	48	−0.1 (−0.3–0.2)	0.33	47	−0.2 (−0.4–−0.0)	<.0001	58	−0.5 (−0.7–−0.3)	<.0001	59	−0.4 (−0.6–−0.2)	<.001
Release Velocity, mm/s	Time 0 to 2 Minutes	48	−57 (−79–−10)	<.001	48	−43 (−77–−28)	<.0001	60	−73 (−118–−54)	<.0001	60	−80 (−99–−39)	<.001
	Time 0 to 5 Minutes	48	−46 (−92–4)	<.001	47	−49 (−93–−22)	<.0001	58	−99 (−130–−56)	<.0001	59	−83 (−124–−45)	<.001

CPR = cardiopulmonary resuscitation; PPE = personal protective equipment; IQR = interquartile range; cpm = compressions per minute.

P-values were obtained from Wilcoxon signed-rank tests.

Table 4.

Change in Fatigue Score Over 2 and 5 Minutes by Provider Group

	Prehospital Provider						Hospital Provider					
	Baseline			PPE			Baseline			PPE		
Time Points Compared	N	Median (IQR)	P-value	N	Median (IQR)	P-value	N	Median (IQR)	P-value	N	Median (IQR)	P-value
Time 0 to 2 Minutes	48	1 (0–1)	<.001	49	1 (0–2)	<.001	64	1 (1–2)	<.001	64	1 (1–2)	<.001
Time 0 to 5 Minutes	49	2 (1–3)	<.001	49	3 (2–5)	<.001	63	4 (2–5)	<.001	64	4 (3–5)	<.001

PPE, personal protective equipment; IQR, interquartile range.

P-values were obtained from Wilcoxon signed-rank tests.