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Kangaroo Care Modifies Preterm Infant Heart Rate Variability in Response to Heel Stick Pain: Pilot Study

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Abstract

Background—Heel stick is the most common painful procedure for preterm infants in neonatal intensive care units. Resultant pain causes adverse physiological effects in major organ systems. Kangaroo Care (KC), involving mother-infant skin-to-skin contact is a promising analgesic for infant pain; however, the effect of KC on the autonomic nervous system's response to pain is unknown.

Aim—To determine if KC results in improved balance in autonomic responses to heel stick pain than the standard method where infants remain in an incubator care (IC) for the heel stick.

Study Design—A randomized cross-over trial.

Subjects—Fourteen preterm infants, 30-32 weeks gestational age and less than 9 days postnatal age.

Outcome Measures—Infant behavioral state, heart rate, heart rate variability (HRV) indices including low frequency (LF) and high frequency (HF) power, and the LF/HF ratio measured over Baseline, Heel Warming, Heel Stick, and Recovery periods in KC and IC conditions.

Results—HRV differences between KC and IC were that LF was higher in KC at Baseline ($p < .01$) and at Heel Stick ($p < .001$), and HF was higher in KC at Baseline than in the IC condition ($p < .05$). The LF/HF ratio had less fluctuation across the periods in KC than in IC condition and was significantly lower during Recovery in KC than in IC ($p < .001$).

Conflict of interest statement: Authors have reported no relevant financial and personal relationships with other people or organizations that could inappropriately influence their work.

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Conclusions—Infants experienced better balance in response in KC than IC condition as shown by more autonomic stability during heel stick. KC may be helpful in mediating physiologic response to painful procedures in preterm infants.

Keywords

Pain; Heel Stick; Kangaroo Care; Heart Rate Variability; Preterm Infants

1. Introduction

Preterm infants are subjected to numerous invasive procedures as part of their care during stays in neonatal intensive care units (NICUs). In several studies, preterm neonates had a mean of 10 to 16 painful procedures per day during their first several days of life; heel sticks were the most common source of pain and most frequently untreated for pain relief [1-7]. Preterm infants can detect, process, and respond to painful stimuli since autonomic ascending pathways for pain transmission develop as early as the 20th week of gestation [8,9]. Simultaneously, infants may actually have a 30-50% lower pain threshold than that of adults and a lower pain tolerance than older children. Thus the preterm infant is at greater risk for pain than a full-term infant due to immaturity of the descending pathway to inhibit or dampen nociception at birth, leading to hypersensitivity to pain [10,11]. Excessive and prolonged unrelieved pain in the infant causes adverse physiological effects in all major organ systems, can be life threatening and can have long-term cumulative outcomes [12,13]. Effective, non-pharmacological interventions are valuable alternatives for pain relief during invasive procedures in neonates [14,15].

Kangaroo Care (KC), also called mother-infant skin-to-skin contact, has been shown so consistently to be an analgesic for procedural pain [16-20] that the American Academy of Pediatrics [21] and others [22-27] recommend KC as an effective non-pharmacologic pain intervention. KC's proposed action as a pain treatment is supported by the Neuromatrix Theory of Pain [28], in which pain is postulated as a multidimensional output produced by a widely distributed neural network in the brain and determined by many factors, such as context, company, competitive stimuli, and meaning [29]. During KC, the mother's skin-to-skin contact with her preterm infant provides multi-sensory stimulation including emotional, tactile, proprioceptive, vestibular, olfactory, auditory, visual, and thermal stimulation in a unique interactive style. When the infant undergoes a heel stick, KC and its multi-sensory inputs may act on the pain matrix programs to modulate and inhibit pain perception, and to contribute to the outflowing neurosignature in such a way that pain responses are minimized. KC's action occurs through multi-sensory input to the brain, activation of the neuro-chemical system, and modulation of the stress-regulation system involved in pain experience [19].

KC has been shown to reduce both physiologic and behavioral responses to pain in preterm infants. Physiologic changes documented have included a decreased variation in heart rate (HR) [16,19,30], a diminished increase in HR [17,18,31], an increased level of oxygenation [30] and increased stability in oxygen saturation [18,32], stability in respiratory rate [32], decreased central venous pressures [30], and a shortened recovery time as indicated by return to baseline physiological values [30,33]. Behavioral changes due to KC have been a decrease in crying time [18,19,34], as well as a decrease in and a shortened duration of facial expressions of pain [16-18,31].

Infants often show differences in behavioral and physiologic responses to pain [35-37]. Behavioral responses may diminish, but physiological responses may remain elevated or increase. Without a reduction of physiological responses, infants' organs remain exposed to adverse effects of pain [35]. Because the autonomic nervous system responds to the environment by increasing (in the stressful environment) or decreasing (in a calming

environment) cardio-respiratory parameters, the most relevant measure of KC's pain reduction ability is assessment of KC's effects on the autonomic nervous system. Heart rate variability (HRV) has been hypothesized to be a sensitive indicator of autonomic function in relation to pain and has been used as a non-invasive measure of parasympathetic and sympathetic reactivity to pain in preterm infants [38-41]. The frequency domain analysis of HRV delineates parasympathetic from sympathetic components of autonomic control, i.e. by using power spectral analysis [42]. The spectral power of the high-frequency (HF) band (0.15 -1.0 Hz) is related to respiratory sinus arrhythmia and reflects parasympathetic activity. The spectral power of the low-frequency (LF) band (0.04 -0.15 Hz) is an index of primarily sympathetic activity with some parasympathetic input [42-45]. HRV is a recommended indicator to be examined in response to a painful event shortly after birth [46]. However, the effect of KC on the HRV response to pain is not known.

Thus, the purpose of this randomized cross-over experimental study was to determine if a heel stick performed in a KC intervention condition showed different autonomic responses to pain than a heel stick in the standard incubator care condition. Pain responses were measured by use of spectral analysis of heart rate variability.

2. Methods

2.1. Design

A prospective cross-over with random assignment by permuted block design was used. A statistician helped the investigator generate a list of randomization codes using the SAS[®] procedure PLAN. The list of random codes consisted of the subject's number and the treatment assignment. According to the random codes, infants were assigned to two groups, determined by the sequence of the KC and current standard of care – routine incubator care (IC) conditions. Group A received routine IC on the first day of the study and KC on the second day. Group B received KC on the first day and routine IC on the second day. Infants served as their own controls. A 24-hour routine IC washout period was incorporated into the design for both groups. Twenty-four-hours was sufficient to allow any lingering effects of KC to dissipate. Previous research has shown that KC effects on cardiorespiratory and behavioral state outcomes disappear within three hours [47-49], and KC's blunting effects on plasma and salivary cortisol were not sustained a day later [50], suggesting that all carry-over effects of the first day's treatment would be absent 24 hours later.

2.2. Subjects

Institutional Review Board approval was obtained and mothers gave written informed consent. Inclusion criteria were infants 30 through 32 weeks gestational age (GA), two through nine postnatal days old, cared-for in an incubator, and whose mothers were English speaking. Exclusion criteria were infants with known congenital anomalies, periventricular/intraventricular hemorrhage (\geq Grade III), history of surgery, having received sedation, vasopressor, or analgesics, exposure to drug abuse during pregnancy, multiples at birth, and showing signs of severe tissue breakdown of either heel as measured by the Neonatal Skin Condition Score [51].

No study was found that measured HRV as an outcome of KC in relation to preterm infant pain responses. Based on Lindh's finding [41] of an increase in low frequency power with $M = .30$, $SD = .35 \log \text{mHz}^2$ in the response of preterm infants to heel stick compared to baseline, as well as medium effect size of KC on pain scores in Johnston's [18] study, power analysis showed that 14 subjects were needed to detect the effect of KC (effect size = 0.40) on HRV, with $\alpha = .05$ and power = .80. Eighteen infants and their mothers were approached in a Level II NICU in a non-profit community hospital located in Washington state and 14 healthy

preterm infants (8 male and 6 female, Group A = 7, Group B = 7) and their mothers were included in the final sample (Figure 1).

2.3. Measures

Infant's behavioral state—The Anderson Behavioral State Scoring System (ABSS) [52] was used to measure infant state. The ABSS has 12 categories with behavioral states of increasing agitation: 1 = regular, quiet sleep; 2 = irregular sleep; 3 = active sleep; 4 = very active sleep; 5 = drowsy; 6 = alert inactive; 7 = quiet awake; 8 = active awake; 9 = very awake; 10 = fussing; 11 = crying and 12 = hard crying. Content and convergent validity of the ABSS have been reported extensively [53,54]. Interrater reliability has been estimated at .71 to .95 in two studies [53,55]. For each assessment, an infant was observed for 30 seconds, and the number corresponding to the highest behavioral state observed was recorded [55].

Heart rate and heart rate variability—Continuous electrocardiogram (ECG) data were recorded from two surface chest electrodes throughout baseline, heel warm, heel stick, and recovery periods. R-R intervals and respiratory activity were captured and measured using the ANS-R1000 system (Ansar, Inc., Philadelphia, PA), a non-invasive signal monitor, which is an accessory connecting to the infant cardio-respiratory monitor that captures ECG and respiratory data on line. The ECG was converted by the computer software to digital values and a time series curve of the R-R intervals was generated. Heart rate and respiratory activity graphs displayed the 128 second (512 points) segment required to produce the HR and respiratory activity spectra. Following smoothing of the curve, the computer re-sampled at regular intervals over the same period using a Fast Fourier Transform (FFT) [56]. Spectral analysis of the transformed data generated two components of clinical interest: the HF (0.15 - 1.0 Hz) component, which is identified with vagal tone and is determined by the respiratory rhythm; and LF (0.04 to 0.15 Hz) component, which is mediated by both sympathetic and parasympathetic parts of the autonomic system. The ratio of the LF/HF spectra represents an index of parasympathetic-sympathetic balance. Movement and artifact were eliminated by comparing amplitude (height) of the R-wave to be included with the amplitude for the last acceptable R-wave. Waves of more or less than 38% deviance from the previous wave were automatically eliminated. The researcher or research assistant who extracted the HRV data was not blinded from the study conditions. Although the bias was likely minimal, still it is important. A proper blinded data extraction process would be necessary to guard against bias pertaining to knowledge of study conditions in the future study.

2.4. Procedures

Based on ethical considerations and pain management guidelines from the American and Canadian Pediatric Societies [21], only heel sticks that were clinically warranted and ordered by physicians or neonatal nurse practitioners were used as the painful procedure in the study. Such procedures are typically ordered on at least a daily basis, as was the case for the neonates in this study. No additional bloodwork was used in the research procedure.

The demographic data and the number of previous invasive procedures were obtained from the patients' medical records. Data collection on infants' responses to pain in KC and in IC was conducted on two consecutive mornings between 8:30 am and 10:20 am. The heel stick and subsequent blood draw were standardized and performed in accordance with the guidelines and step-by-step procedure developed by National Association of Neonatal Nurses [57]. A Tenderfoot™ device was used to lance the heel. One consistent person, the neonatal unit phlebotomist, did all the heel sticks and blood draws. In both KC and IC conditions, data were collected across four periods: (1) **Baseline (BL)**, 20 minutes immediately prior to heel warming, (2) **Heel Warming (HW)** with a warm pack, 5 minutes, (3) **Heel Stick (HS)**, 15

seconds, and blood collection with possible further squeezes, 0.5 to 10 minutes, and (4) **Recovery (RC)**, 20 minutes after a band-aid was placed on heel.

Procedure for KC Condition—The infant was transferred from the incubator into KC after a naso-gastric tube or bottle feeding was completed. KC was carried out with the mother holding her preterm infant prone, clothed only in a diaper, skin-to-skin, between her breasts. All infants were held upright at a 30 – 40 degree angle. The infant's back was covered with a receiving blanket folded in fourths and placed beneath the mother's cover gown to insure infant temperatures were sustained within a neutral thermal zone. Mother and infant were seated in a recliner next to the infant's incubator. Mothers were encouraged to rest during KC, but could talk softly. Mothers were encouraged to leave the infant alone if he/she was sleeping and to leave their hands clasped behind the infant's back. Mothers and infants had 60 minutes of undisturbed KC before heel warming began. Heel Warming, then Heel Stick, and then the Recovery period were executed in the KC position.

Procedure for IC condition—At the end of the morning feeding, the diaper-clad infant was placed prone and nested in a servo-control incubator inclined at 30°-40°. Mothers were absent during the incubator periods. Infants remained undisturbed for 60 minutes, and then Heel Warming, Heel Stick, and Recovery periods were executed while the infant remained in the incubator in the same position.

During both the KC and IC conditions, one of three research nurses observed and coded behavioral states every minute during the four data collection periods. These nurses were trained in the behavioral state assessment procedures before the study. Assessed inter-rater reliability (Cohen's kappa) among the three research nurses was 95% at the beginning of the study and was maintained at 91%-95% agreement during the study by reestablished after every 5th participant. HRV data were collected during four periods in both the KC and IC conditions.

2.5. Data analysis

Infant behavioral states were described as the percentage of time in each state for each of the data collection periods (i.e. BL, HW, HS, and RC). The HRV indices were not significantly different among the behavioral states, and so the HRV data were combined for each data collection period; arithmetic means were calculated for HR and geometric means were calculated for HRV indices for each of the four data collection periods in the KC and IC conditions. The geometric mean $[GM = (a_1 \cdot \dots \cdot a_n)^{1/N}]$ was used instead of the arithmetic mean $[M = (a_1 + \dots + a_n)/N]$ for calculating HRV indices, because the GM results in an average rate of change [58]. Repeated measures analysis of variance (RM-ANOVA) was used to compare HR and HRV indices across BL, HW, HS, and RC periods using SPSS 13.0 (Chicago, IL). To address possible carry-over effects and dependence of observations within subjects, the Generalized Estimating Equations (GEE) model analyzed treatment (KC vs. IC) and carry-over effects (assignment to KC day 1 vs. KC day 2) using SAS 8.0 (Cary, NC). The logarithm transformation was applied before the analyses, because the HRV data were not normally distributed.

3. Results

3.1. Characteristics of subjects

Eighteen infant-mother dyads were approached and 16 were enrolled (Figure 1). After randomized allocation, one subject was withdrawn prior to any treatment administration and data collection and one infant missed HRV data in both IC and KC days due to equipment issues. Fourteen subjects included in the final sample. Seven were randomly assigned to group A (IC Day 1, KC Day 2) and seven to group B (KC Day 1, IC Day 2). Because no statistical

differences between the groups were found on demographic and medical characteristics, and because all babies served as their own controls, the data are presented for all participants as one group. Demographic and medical characteristics are in Table 1. The mean number of previous painful procedures was 29.15 ± 6.44 with a range of 20 - 40. Previous painful procedures were heel sticks, intubation, placement of an umbilical line or a percutaneous venous catheter or peripheral intravenous lines, arterial punctures, lumbar puncture, chest tubes, urinary catheterization for bladder tap, and vitamin K injections. Heel stick was the most frequently performed previous painful procedure, constituting a mean of 16 out of the 29 (54%) of the procedures, and every baby had at least one heel stick procedure during their NICU stay.

3.2. Behavioral state

During BL, neonates were in Quiet Sleep 65% of the time in KC and 60% in IC, and in Active Sleep 24% of the time in both KC and IC. During HW, neonates were in Quiet Sleep 65% of the time and in Active Sleep 22% of the time in both KC and IC. During HS, most neonates cried (65% in KC; 64% in IC). During RC, neonates were predominantly in Quiet Sleep (57% in KC, 48% in IC) or Active Sleep (21% in KC, 25% in IC). No significant differences in behavioral states were found between KC and IC conditions during the BL, HW, HS, and RC periods.

3.3. Heart rate and heart rate variability indices

Heart rate and HRV data were available for all days except one KC and four IC days due to equipment problems. The pairwise deletion was used for missing data; therefore, the final data were from 13 babies in KC and 10 babies in IC. To test infants' pain responses for the heel stick across the four study periods (BL, HW, HS, and RC), individual RM-ANOVA with study periods as the repeated factor was conducted for each study condition (KC and IC). HR increased significantly during HS from the BL and HW periods in both KC ($p < .05$) and IC conditions ($p < .001$), and returned to BL values during RC in both conditions (Table 2 and Figure 2.A). Comparing HR between KC and IC heel stick conditions using the GEE model, HR was significantly lower in the KC condition (146 ± 9 beats/min) than in IC (152 ± 13 beats/min) during BL period ($p < .05$) and HS period (KC 159 ± 14 beats/min vs. IC 165 ± 14 beats/min, $p < .05$).

There were significant differences in HRV indices across BL, HW, HS, and RC periods in both KC and IC conditions using RM-ANOVA with study periods as the repeated factor, and the differences followed similar patterns in both study conditions (Table 2; Figure 2.B, 2.C, and 2.D). Both LF and HF were increased during HS from the BL and HW, and dropped in the RC period in both KC (LF, $p < .05$ and HF, $p < .01$) and IC (LF, $p < .01$ and HF, $p < .001$) conditions. The LF/HF ratio was lower during HS than during BL, HW, and RC in both KC ($p < .01$) and IC ($p < .001$) conditions. When testing the KC effect on the heel stick compared to the IC condition using the GEE model, HRV differences between KC and IC indicated that LF was higher in KC at BL ($p < .01$) and at HS ($p < .001$) than in IC, and HF was higher in KC at BL than in IC ($p < .05$). The LF/HF ratio was significantly lower during RC in the KC than IC condition ($p < .001$). Results showed medium effect size (0.35 to 0.40) of KC on modifying HRV indices during heel stick procedure.

4. Discussion

In this study, the patterns shown over time in the HR and HRV indices provided a noninvasive measure of sympathovagal balance during heel stick among 14 preterm infants 30 – 32 weeks GA in KC and IC conditions. To our knowledge, the investigation reported here is the first study examining the effect of KC on heel stick pain responses measured by HRV indices in preterm infants. Consistent with previous studies [41,59-61], HR increased from baseline to

heel stick and decreased in the recovery period in both the KC and IC conditions, indicating a clear pain response caused by the heel stick procedure. The LF and HF responses were also found similar in both KC and IC with increases from BL to HS and decreases from HS to RC. In relation to painful events in preterm infants, previous studies showed that total HRV [41], the LF [41,59] and HF bands of HRV [59] were reduced during heel lance, but other studies did not show the correlation of HRV to pain [38,39]. Our sample differed in the LF and HF responses with increases at heel stick instead of decreases as reported in these previous studies. The reason for this difference is not clear as our infants' positions and behavioral responses were similar to those in other studies. It may be that the length of the data collection period during Heel Stick varied among the studies so that the intervals are not comparable. In our study, data were collected across the HS period which ranged from 3.5 – 4.5 minutes; the length of data collection was not reported in the two Oberlander studies. The LF/HF ratio response in our sample was the same as that reported by Oberlander and colleagues [59,60] with a decrease in the LF/HF ratio at heel stick. In both KC and IC, the LF/HF ratio decreased from BL to HS, and increased in RC, reflecting an increase in parasympathetic influence in order to balance sympathetic response to the heel stick.

When comparing infants' pain responses between KC and IC conditions, HR was significantly lower in KC than IC for the BL and HS periods. LF and HF were higher in the BL period and LF was also increased in the HS period in KC compared to the IC condition with a medium effect size (Figure 2.B and 2.C). The results are consistent with Schrod and Walter's [62] findings, in which LF and HF increased when infants were tilted into the KC position. Another study by Begum and associates [63] found that LF was significantly increase while HF was decreased during KC in low birth weight infants. However, none of these previous studies tested the effect of KC on HRV in a procedural pain condition. Increases in LF (predominately sympathetic tone) and HF (parasympathetic tone) may be explained by several factors, i.e., maturation (gestational age and postnatal age), change in body position, sleep state, and maternal presence and body temperature [44, 62, 64, 65]. In the present cross-over design study, infants in both KC and IC condition have equivalent GA and postnatal age, similarly inclined 30 – 40 degree prone position, and no difference in behavioral states during the study periods. One explanation for higher LF and HF in KC than in IC may be maternal presence and touch. Changes in LF have been found in response to thermoregulation influences and stimulation in preterm infants. Infant sympathetic activity is increased in a mother-infant bed-sharing environment compared to solitary-sleeping, which might be partly explained by thermal stimulation and thermoregulation when mothers are present [66]. Increased environmental air temperature due to the mother's body temperature also causes LF to rise in KC. The most likely explanation is that increased environmental temperature in KC increased infants' central temperature, thus causing a concurrent increase in LF compared to IC. KC also activates pressure receptors that can increase parasympathetic activity. Studies of moderate-pressure massage therapy show that parasympathetic activity peaks during massage and remains significantly higher throughout the 15-minute post-massage period compared with infants who receive sham, light-pressure massage [67, 68]. Animal studies also indicate that tactile interactions between rat pups and their mother, a type of pressure receptor stimulation, activates pups' parasympathetic responses and prevents all the changes associated with maternal deprivation [69-71]. Increased HF in KC may be due to KC's stimulating infants' pressure receptors, because the pressure receptors located in an infant's chest, abdomen, and extremities are activated by the full body touch between the infant and mother in KC, and the intensity of pressure in KC may be similar to moderate pressure.

For LF/HF ratio, there was more stability in KC and greater fluctuations in IC across BL, HW, HS, and RC periods (Figure 2.D). The ratio was significantly lower in KC than in IC condition during the RC period with a medium effect size. A more mature, balanced response (lower LF/HF ratio) to the painful heel stick during recovery was present in KC compared to that in IC.

Balance between parasympathetic and sympathetic tone is associated with maturation and the ability to react effectively to stress, such as heel stick pain. Sympathetic tone is dominant in preterm infants, whereas parasympathetic tone increases with maturation [64, 72]. A lower LF/HF ratio indicates more maturity and a greater balance between the two systems [73]. The findings suggest a more balanced, and stable autonomic response to a painful heel stick in KC compared to that in IC. The fluctuations in autonomic response in IC were due to greater swings in sympathetic activity, suggesting that KC has a stabilizing, or balancing, effect during a painful procedure such as heel stick.

The central underlying mechanisms of KC as an analgesic and comforting experience on reducing infant pain may be through multi-sensory stimulation inputs and modulation of the stress-regulation system involved in pain. Mother-infant physical contact has been found to trigger release of beta-endorphins that are critically involved in mediation of the pain signal by blocking the perception of pain in rat pups [74,75] and in human newborns [76]. Dieter [77] and Harrison [78] suggested that systematic gentle human touch may stimulate peripheral nerves that activate the vagus nerve, thus promoting infant comfort and reducing stress, resulting in positive immediate and long term outcomes. In addition, KC compared to the incubator condition provides stimulation through the infant's prone positioning [79-83], maternal warmth [84], containment and swaddling [85-87], maternal heart sounds [88,89], vestibular movement (mother's chest respiratory movement) [90], maternal body odor [91-93], and mother's voice [94]. Infants are apparently familiar with their mothers' odor, voice, respiratory and heart beat rhythm from the uterine environment [92] and these have soothing effects on infants. Following KC, preterm infants showed a more rapid maturation of vagal tone as compared to controls, underscoring the effect of KC on the autonomic and circadian systems in preterm infants [19,58,95]. These findings support the premise that maternal effects reduce infant pain by blunting sympathetic responses, accelerating parasympathetic recovery of autonomic activation, and activating the endogenous opioid system.

These HRV findings of better balance and autonomic stability in the Kangaroo Care condition during heel stick for preterm infants lend further support the findings about KC decreasing HR, crying, and grimacing during painful procedures [16-19,31,34,96-98]. Our study's findings add to the continuing evidence for KC as a non-pharmacologic intervention to alleviate preterm infant pain responses related to the heel stick.

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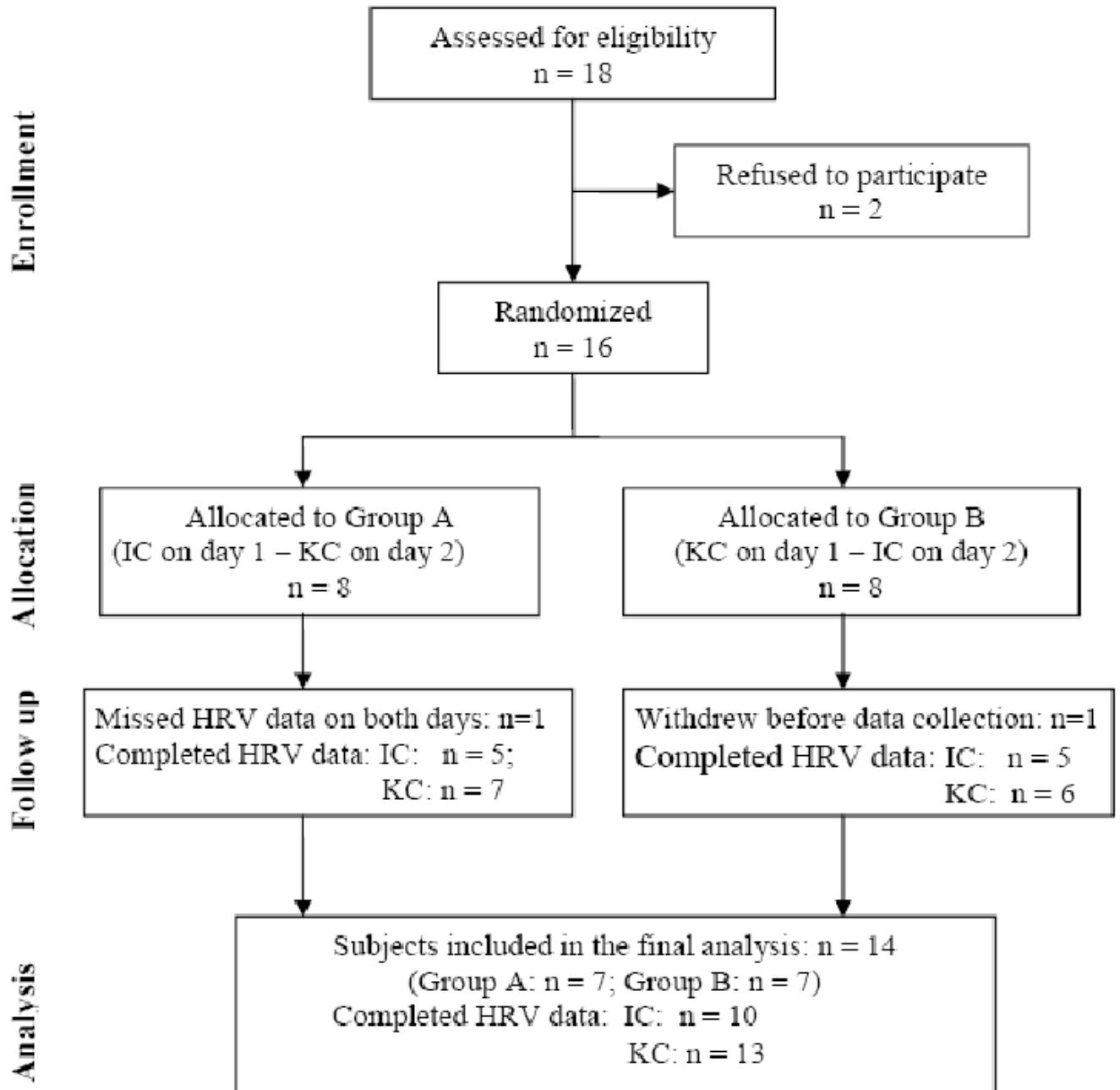
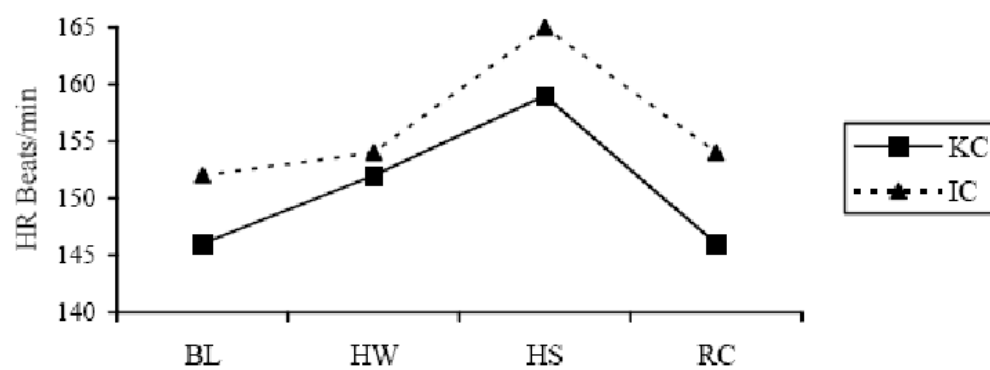
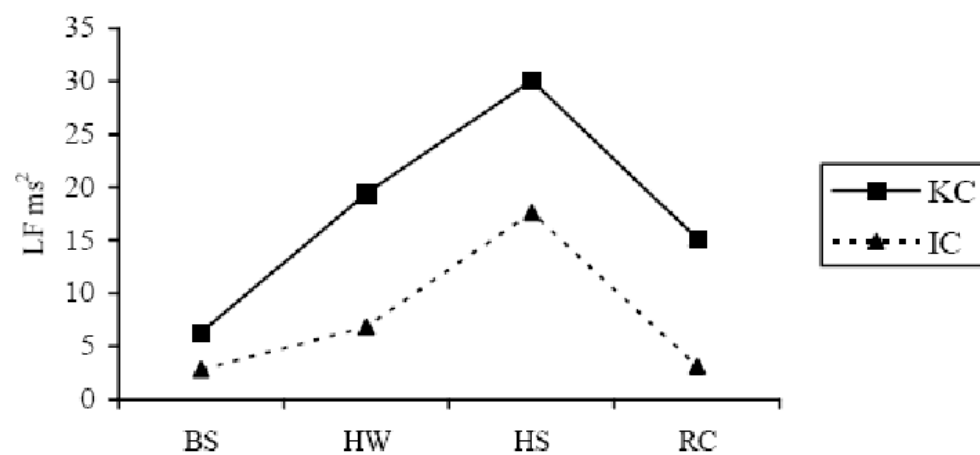


Figure 1. Consort diagram of enrollment, allocation, follow-up, and data analysis. KC = Kangaroo Care heel stick condition; IC = Incubator heel stick condition.

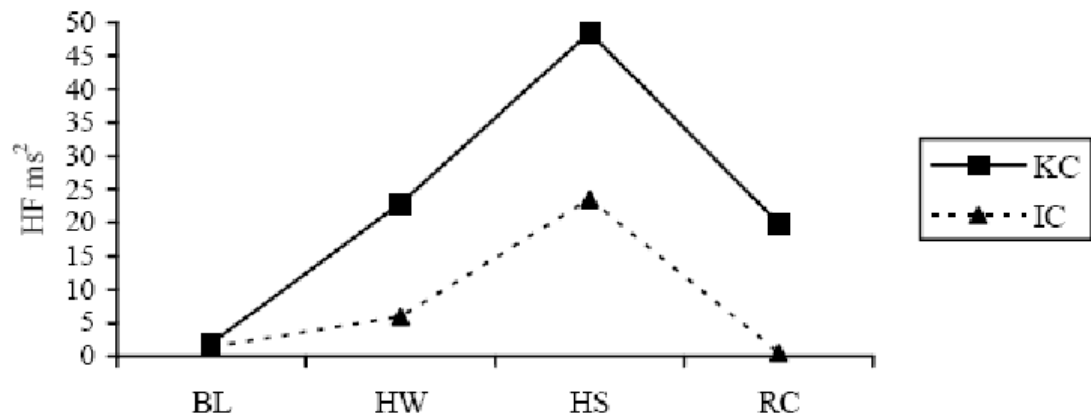
2.A. Heart Rate



2.B. Low Frequency Power



2.C. High Frequency Power



2.D. Low/High Frequency Power Ratio

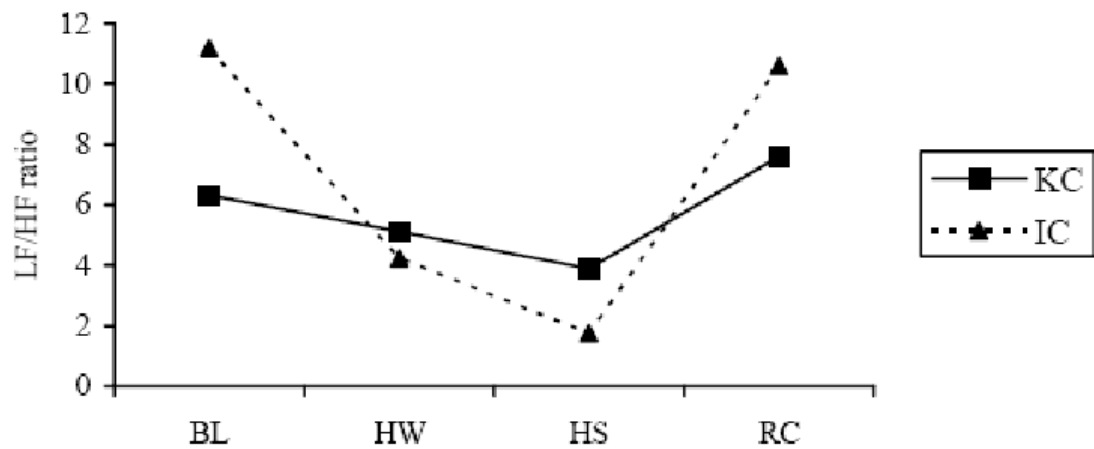


Figure 2. HRV indices across four periods in the KC and IC conditions. KC = Kangaroo Care condition; IC = incubator care condition; LF = low frequency power (predominately sympathetic activity); HF = high frequency power (parasympathetic activity); LF/HF = Low/High frequency power ratio (balance of sympathetic-parasympathetic activity).

Table 1
Demographic and Medical Characteristics of Infants (n = 14)

Variables		Values
Gestational age:	30 weeks	5 (36%)
	31 weeks	3 (21%)
	32 weeks	6 (43%)
Gender:	Male	8 (57%)
	Female	6 (43%)
IVH incidence:	None	13 (93%)
	Grade I	1 (7%)
Race/Ethnicity:	Caucasian NonHisp	7 (50%)
	NonCauc Hispanic	7 (50%)
Postnatal age (days)		6 ± 1
Birth weight (g)		1775 ± 292
Weight on the day of study (g)		1706 ± 293
1 minute APGAR score		7 ± 2
5 minutes APGAR score		8 ± 1
Number of previous pain experiences		29 ± 7

Note: IVH= intraventricular hemorrhage

Table 2
Values for HR, LF, HF, and LF/HF during Four Periods in KC and IC Conditions¹

	KC (n = 13)		IC (n = 10)		Z	df	p-value
	M	SD	M	SD			
HR (beats/min)							
BL	146.46	9.36	152.07	12.83	2.20	1 (19)	< .05
HW	152.10	11.65	153.71	11.91	0.11	1 (18)	> .05
HS	158.64	13.58	164.87	14.48	2.43	1 (16)	< .05
RC	146.42	14.17	153.86	14.12	1.44	1 (18)	> .05
Comparison across 4 periods ²	F _(3, 12) = 4.43, p < .05		F _(3, 9) = 12.16, p < .001				
LF (ms²)							
BL	6.30	10.38	2.83	2.65	2.59	1 (19)	< .01
HW	19.44	54.37	6.84	6.38	0.60	1 (18)	> .05
HS	30.05	41.26	17.62	24.55	3.43	1 (17)	< .001
RC	15.16	29.85	3.13	2.46	1.46	1 (18)	> .05
Comparison across 4 periods ²	F _(3, 10) = 4.06, p < .05		F _(3, 9) = 4.75, p < .01				
HF (ms²)							
BL	2.05	3.77	1.38	3.10	2.00	1 (19)	< .05
HW	22.91	70.57	5.96	8.62	1.12	1 (18)	> .05
HS	48.50	71.04	23.52	35.96	1.24	1 (17)	> .05
RC	19.89	54.42	0.50	0.52	1.71	1 (18)	> .05
Comparison across 4 periods ²	F _(3, 10) = 6.18, p < .01		F _(3, 9) = 10.88, p < .001				
LF/HF ratio							
BL	6.32	4.56	11.18	9.00	0.77	1 (20)	> .05
HW	5.12	5.71	4.23	4.90	0.95	1 (18)	> .05
HS	3.89	6.69	1.75	1.84	0.07	1 (17)	> .05
RC	7.61	5.26	10.60	8.63	3.59	1 (18)	< .001
Comparison across 4 periods ²	F _(3, 10) = 6.07, p < .01		F _(3, 9) = 11.63, p < .001				

¹ HR = arithmetic mean, LF, HF, L/HF = geometric means;

² Comparison across 4 periods: Repeated measures analysis of variance was used;

³ Comparison KC vs. IC: Generalized Estimating Equations model was used; KC = Kangaroo Care condition; IC = Incubator care condition; BL = Baseline; HW = Heel Warming; HS = Heel Stick; RC = Recovery; LF = low frequency power; HF = high frequency power; LF/HF = Low/High frequency power ratio.

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