Title

Kangaroo mother care alters chromogranin A and perfusion index in preterm babies

Author(s)

Tomohiro Endo, Mari Sampei, Seiji Fukuda

Journal

Pediatrics international : official journal of the Japan Pediatric Society 63(1):53-59

Published

16 June 2020

URL

https://doi.org/10.1111/ped.14350

Original Article

Kangaroo Mother Care Alters Chromogranin A and Perfusion Index in Preterm Babies

Tomohiro Endo, MS [1], Mari Sampei, PhD [1, 2], Seiji Fukuda, MD, PhD[1, 3, 4]

1: Department of Clinical Nursing, Shimane University Faculty of Medicine

2: Fukushima Medical University, School of Nursing

3: Department of Pediatrics, Shimane University Faculty of Medicine

4: Division of Patient Safety, Shimane University Hospital

89-1 Enya, Izumo, Shimane, 693-8501, Japan

Running title: Effect of Kangaroo Mother Care on salivary CgA

Address correspondence to:

Seiji Fukuda, MD, PhD; sfukuda@med.shimane-u.ac.jp

Tel: +81-853-20-2718, FAX: +81-853-20-2317

Number of text pages: 19, Word count: abstract 224, text excluding title page, references and figure legends: 2875, reference pages: 4, number of references 43, number of tables: 2, number of figures: 3, Legends to figures: 3

ABSTRACT

Background: While providing various benefits, concerns regarding the potential risks of kangaroo mother care or skin-to-skin contact (SSC) between mother and child for preterm infants hamper its widespread implementation in some advanced countries. Salivary chromogranin A (s-CgA) is elevated upon exposure to stress, whereas the perfusion index (PI) is associated with hemodynamics and peripheral perfusion in neonates. Herein, we investigated the effects of SSC on s-CgA and the PI in preterm infants.

Methods: Twelve infants were enrolled. Factors associated with baseline s-CgA were analyzed. Baseline s-CgA and the level after SSC were compared. Secreted IgA in the saliva was compared as the control. The PI before, throughout and after SSC were compared.

Results: The baseline CgA was significantly lower in infants who were supplemented with baby formula milk in addition to breast milk before SSC (n=2) compared with those fed with their mother's breast milk alone (n=10, P=0.03). SSC significantly decreased s-CgA in babies who were fed breast milk only before SSC (n=10, P<0.01) but not in those supplemented with formula milk before SSC (n=2). Secreted IgA in saliva was not affected by SSC. The PI was significantly elevated during SSC (P<0.01).

Conclusion: Our data indicate that SSC can reduce s-CgA levels when combined with mother's breast milk and increase the PI in preterm infants, thereby providing additional evidence of the benefit of SSC.

Keywords: kangaroo mother care, perfusion index, preterm infants, salivary chromogranin A, skin-to-skin contact

INTRODUCTION

The number of infants born preterm, which is generally associated with increased mortality and morbidity, is increasing (1, 2). Kangaroo mother care was originally developed as a practice of skin-to-skin contact (SSC) between infants and mothers in countries where appropriate medical resources for preterm neonates are unavailable (3). SSC decreases mortality (4), bronchopulmonary dysplasia (5), and infectious illness (5, 6) in neonates. SSC also increases body weight (6, 7) and is associated with greater physical development in preterm infants (8). Although several studies have provided evidence of favorable outcomes associated with SSC for preterm infants and their mothers (4, 5, 7, 9-16), no data are available to determine an association between intermittent SSC and decreased mortality in resource-rich countries (16). Moreover, earlier reports have demonstrated rare life-threatening events during SSC (17, 18) and an association between infant body temperature and the increase in the number of apnea, bradycardia and desaturation events during SSC (19-21). In parallel with these findings, SSC is not necessarily widely propagated due to concerns about the infrequent adverse effects in some advanced countries (22, 23). These findings imply that providing additional proof of the benefits of SSC in preterm and/or low-birth-weight infants would facilitate its implementation.

A previous study indicated that SSC decreases salivary cortisol levels, a marker of psychological stress, and increases salivary oxytocin levels in stable preterm infants and their parents (24), suggesting that SSC produces a positive effect in infants by eliminating stress. Chromogranin A (CgA) is a protein secreted along with catecholamines from the adrenal medulla and sympathetic nerve endings (25-27). Salivary CgA secreted from the submandibular glands is a psychological stress marker that increases upon exposure to stress and declines after release from psychological stress (28). Salivary CgA levels increase upon waking in the morning in adults and then dramatically decline and remain relatively stable thereafter during the day (29); by contrast, cortisol continues to decline over the course of the day as part of the circadian rhythm (29, 30). The circadian rhythm of salivary cortisol in infants is already established by one month of age (31). This raises the possibility that the reduction in salivary cortisol following SSC in earlier studies might have reflected the physiological circadian decrease. Another study indicated that salivary cortisol may not always decrease in preterm infants after SSC (32). An elevation in salivary CgA followed by an increase in salivary cortisol upon psychological stress (28) suggests that salivary CgA can be a more sensitive indicator of stress than cortisol. These findings imply that salivary CgA levels may represent an additional surrogate to evaluate stress in preterm

infants and, therefore, would be reduced upon SSC. Although salivary CgA has been extensively studied in adults, little is known about salivary CgA, including its kinetics before and after SSC, as well as factors associated with salivary CgA, in neonates.

SSC was reported to maintain body temperature (21), oxygen saturation, and cardiorespiratory rhythm in infants (6). However, it remains unknown whether SSC stabilizes hemodynamics and peripheral perfusion. The perfusion index (PI), a ratio of the pulsatile and nonpulsatile signals derived from the amount of near-infrared light absorbed by a pulse oximeter, is correlated with peripheral perfusion, cardiac output and the stroke volume (33-35). It predicts the severity of neonatal illness, including congenital heart disease (36) and low superior vena cava flow (37). Lower PI values indicate reduced systemic perfusion (38), and a low PI on day 1 is associated with adverse outcomes in extremely preterm infants (39). By contrast, the PI increases as hemodynamics stabilize in preterm infants (40). Crosstalk between the autonomic nervous system and blood vessels has been demonstrated. Acetylcholine is released from parasympathetic nerves and induces vasorelaxation by regulating the release of NO in arteries through M3 acetylcholine receptors on the endothelium (41). SSC may enhance peripheral perfusion through vasorelaxation by releasing acetylcholine from

parasympathetic nerves. These findings led to the hypothesis that SSC enhances PI in infants.

To determine additional beneficial effects of SSC, we compared salivary CgA and PI in preterm infants before and after intermittent SSC. The specific aims of the present study were as follows:

1) to investigate factors associated with salivary CgA in preterm infants
2) to investigate whether SSC decreases salivary CgA in preterm infants
3) to investigate whether SSC increases PI in preterm infants

SUBJECTS AND METHODS

This study was performed at the neonatal intensive care unit (NICU) of the single public institute of Shimane Prefecture, Japan, between April 2017 and January 2019. Among the infants hospitalized in the NICU, the subjects who met the following enrollment criteria were recruited:

Inclusion criteria:

1. Birth weight less than 1,500 grams
2. Body weight greater than 1,000 grams
3. More than 72 hours after tracheal extubation
4. Approval by chief physician
5. Guardians' agreement to enroll in the study (indicated by written informed consent)

Exclusion criteria

1. Apparent congenital anomalies

During the study period, 8 girls and 4 boys met these enrollment criteria. The average gestational age and birth weight were 28.1±2.6 weeks and 991.7±347.6 g, respectively. The infants were 56.3±23.3 days old with an average body weight of 1,853±178.2 g on the day of the study (Table 1). Six of them were on noninvasive

respiratory support on the day of the examination; however, none were inhaled with oxygen.

Figure 1 illustrates the analysis procedure. In this neonatal ward, the length of SSC is not usually restricted; therefore, the duration of SSC for each subject varied in the present study (median, 108 minutes; range, 70–188 minutes) at the discretion of the infant's mother. SSC was initiated at least 1 hour after feeding. Ten infants were fed only their mother's breast milk, whereas two infants were fed formula milk in addition to their mother's milk before SSC. All the infants contacted their mother's breast in a downward-facing position during SSC. Salivary CgA was obtained by collecting saliva using a cotton swab. The cotton swab was gently inserted into the infant's mouth before and immediately after SSC for 3 minutes to ensure that sufficient saliva was collected, but the infants were not stressed by the procedure. None of them cried while saliva was being harvested. Secretary IgA (s-IgA) in saliva was measured simultaneously as a control. Salivary CgA and s-IgA were quantitated by ELISA at Yanaihara Institute Inc. (Fujinomori, Shizuoka, Japan) through a fee-based service. Oxygen saturation, the pulse rate and the perfusion index (PI) of the infants were continuously recorded using a pulse oximeter (NIHON KOHDEN, Tokyo, Japan) before, throughout and after SSC. The PI

was measured as the pulse amplitude index, which is indicated by $(A - B)/\left\{\frac{A+B}{2}\right\}X100$, where A and B represent pulsatile and nonpulsatile signals, respectively. Each infant's sleep state was evaluated by the mother after SSC based on the score calculated using the Neonatal Behavioral Assessment Scale (NBAS).

Statistical analysis

Baseline- and post-SSC CgA or baseline- and post-SSC s-IgA were compared using the Wilcoxon signed-rank test. Baseline CgA or post-SSC CgA was compared between boys and girls, the presence and absence of respiratory support, the type of nutrition (mothers' milk and formula milk), and sleep state (state 1 and 2) using the Mann–Whitney U test. A P-value less than 0.05 was considered significant. All the data were analyzed using SPSS Statistics for Windows (IBM, Armonk, NY, USA).

Ethics considerations

All the participants were informed about the procedures, the unlikely but potential risks related to collecting saliva, and the significance of the study. Written informed consent was obtained from the mother or family members before enrollment in the study. The study was performed according to the ethical standards of the 1964

Declaration of Helsinki and its later amendments and was approved by the ethics committee of Shimane University School of Nursing (approval number: 298) and Shimane Prefectural Hospital (R17-007).

RESULTS

Factors associated with baseline salivary CgA

The median baseline salivary CgA (s-CgA, before SSC) was 35.9 (range, 2.81–76.5; N=12) pmol/mL. We determined whether the baseline s-CgA differed by age, gender, gestational age, birth weight, body weight, respiratory support, total length of prior SSC, feeding volume per day, and type of nutrition. The baseline s-CgA was significantly lower in infants who were fed baby formula milk in addition to breast milk before SSC (median, 5.0; n=2) than in those supplemented with their mother's breast milk alone (median, 38.3; n=10; P=0.03; Figure 2A). The baseline s-CgA did not differ by age (days old), gender, gestational age, birth weight, body weight, respiratory support, total prior SSC time, and feeding volume per day.

Salivary CgA in infants before and after SSC

Because the type of feeding significantly affected the baseline s-CgA in the subjects, the effect of SSC on s-CgA was analyzed separately in the subjects who were fed with mother's milk only and in those supplemented with formula milk. The post-SSC s-CgA was significantly downregulated compared with the baseline s-CgA in the babies who were fed only breast milk before SSC (Figure 2B; n=10; P=0.009). By

contrast, the post-SSC s-CgA was not significantly different compared with the baseline s-CgA in the babies supplemented with formula milk in addition to their mother's milk before SSC (Figure 2C; n=2; P=0.18). The post-SSC s-CgA/baseline s-CgA ratio was significantly lower in the infants provided with breast milk alone than in the subjects supplemented with formula milk before SSC (Table 2; P=0.03) but was not influenced by age, gender, gestational age, birth weight, body weight, respiratory support, total prior SSC time, and feeding volume per day. The baseline s-IgA (median, 42.9; range, 20.5–121.0) and post-SSC s-IgA (median, 41.4; range, 22.0–135.3) showed no significant difference regardless of the type of nutrition.

Factors associated with post-SSC s-CgA

A comparison of the post-SSC s-CgA based on subgroup factors demonstrated that the post-SSC s-CgA was significantly lower in the infants with respiratory support than in those without respiratory support (22.0±5.1 and 40.3±5.5, respectively; n=6; P=0.026), although the post-SSC s-CgA demonstrated no significant difference compared with the baseline s-CgA in both groups regardless of respiratory support. The post-SSC s-CgA in the infants who were fed breast milk alone and in those who were fed formula milk was not significantly different.

Perfusion index in infants before, during and after SSC

The perfusion index was significantly elevated during SSC (median, 1.3; range, 0.9–1.5) compared to before SSC (median, 0.8; range, 0.5–1.0) and after SSC (median, 0.6; range, 0.4–0.9) ($P<0.01$; Figure 3A). By contrast, the oxygen saturation and pulse rate did not change before, during and after SSC (Figure 3B and 3C).

DISCUSSION

The present study indicated that the baseline s-CgA was significantly decreased in the preterm infants who were supplemented with formula milk in addition to their mother's breast milk compared with those who were fed only breast milk before SSC, indicating that formula milk modulates the s-CgA level. Comparison of baseline and post-SSC s-CgA demonstrated that SSC significantly decreased s-CgA in preterm infants who were given only their mother's breast milk before SSC. By contrast, SSC failed to reduce s-CgA in the infants supplemented with formula milk. These data suggest that SSC in combination with breast milk, but not with formula milk, decreases s-CgA, implying that SSC in combination with mother's breast milk decreases infant stress. The present study also demonstrated that SSC significantly increased the PI in preterm infants during SSC, providing additional evidence that SSC provides favorable effects on preterm infants in the neonatal intensive care environment.

The baseline s-CgA was significantly lower in the infants who were supplemented with formula milk in addition to breast milk than in subjects who were supplemented with their mother's breast milk alone, suggesting that adding formula milk resulted in a reduction in s-CgA. In this regard, formula milk itself may be

beneficial to decrease s-CgA in preterm infants, although this idea needs to be validated by additional studies involving a large number of subjects. Although a significant reduction in s-CgA was observed following SSC exclusively in the subjects who were fed with only breast milk ($P<0.01$; $n=10$), an identical decrease was no longer detected in those receiving formula milk. The lack of an effect of SSC on s-CgA in those fed with formula milk is likely because the effect of formula milk predominates and/or abrogates the effect of SSC. These findings suggest that the combination of breast milk and SSC will lead to better consequences than either one of them alone in preterm infants. Alterations in s-CgA before and after SSC without changes in control s-IgA levels suggest that the reduction of s-CgA is a specific consequence by SSC but does not represent a universal decline in infants' salivary protein.

A recent study demonstrated that longer SSC is beneficial for premature neonates (5). While SSC significantly decreased s-CgA in the infants who were given only their mother's milk, the duration of SSC showed no correlation with the change in s-CgA in these subjects. It is plausible that moving infants from the incubator to the prone position on their mother's breast can cause stress. If this is the case, s-CgA levels would increase shortly after the initiation of SSC. However, a previous study indicated

that SSC decreased salivary cortisol in as little as 20 minutes in clinically stable preterm babies (42). Another study demonstrated that 60 minutes of SSC decreased salivary cortisol in stable preterm infants (24). Nevertheless, the kinetics of s-CgA levels throughout SSC and the ideal length of SSC providing the most favorable outcomes warrant further investigation.

Finally, our study demonstrated that SSC increases the perfusion index (PI) during SSC. Changes in the PI are associated with alterations in the stroke volume, vasomotor tone and skin temperature, and the PI is an indirect, noninvasive measure of peripheral perfusion. Lower PI values represent reduced systemic perfusion and can indicate various conditions associated with illness in preterm infants (38). The significant increase in PI in preterm infants during SSC suggests that SSC has favorable effects on peripheral perfusion and other physiological functions related to hemodynamics in these infants. Crosstalk between the autonomic nervous system and blood vessels has been demonstrated. SSC may enhance peripheral perfusion through vasorelaxation by activating parasympathetic nerves. Additionally, SSC did not influence oxygen saturation and the pulse rate, indicating that SSC is not associated with adverse events in the present study.

One limitation of this study is the lack of a control group—namely, a group lying at rest without SSC—raising the possibility that s-CgA may decline regardless of SSC. However, a previous report indicated that SSC decreases salivary cortisol levels that did not change upon solitary rest in full-term infants (43). Moreover, s-CgA levels remain low and relatively consistent during the day after the morning surge in adults (29). SSC in the present study was performed during the day to avoid circadian effects on s-CgA. This makes it unlikely that staying at rest per se induces a decline in s-CgA levels during the day, although circadian changes in s-CgA levels in preterm infants remain to be determined.

In conclusion, the present study showed that SSC can decrease s-CgA levels when combined with breast milk and increase the PI, strongly suggesting that SSC decreases stress and favorably affects hemodynamics in preterm infants. Although additional studies are warranted to determine the factors influencing s-CgA levels, our findings imply that salivary CgA levels may represent an additional surrogate to evaluate stress in preterm infants.

ACKNOWLEDGMENTS

The authors are grateful to all participants and their physicians who willingly contributed to the analyses. The study was supported by research support funds from Shimane University (to SF and MS).

DISCLOSURE

The authors declare no conflicts of interest related to this study.

AUTHOR CONTRIBUTION

TE designed the projects, collected the data, analyzed the data and wrote the manuscript. MS designed the projects, and SF directed and designed the projects, analyzed and interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

REFERENCES

1. Ahmed AH, Sands LP. Effect of pre- and postdischarge interventions on breastfeeding outcomes and weight gain among premature infants. Journal of obstetric, gynecologic, and neonatal nursing : JOGNN. 2010;39(1):53-63.
2. McCabe ER, Carrino GE, Russell RB, Howse JL. Fighting for the next generation: US Prematurity in 2030. Pediatrics. 2014;134(6):1193-9.
3. Charpak N, Ruiz JG, Zupan J, Cattaneo A, Figueroa Z, Tessier R, et al. Kangaroo Mother Care: 25 years after. Acta Paediatr. 2005;94(5):514-22.
4. Lawn JE, Mwansa-Kambafwile J, Horta BL, Barros FC, Cousens S. 'Kangaroo mother care' to prevent neonatal deaths due to preterm birth complications. International journal of epidemiology. 2010;39 Suppl 1:i144-54.
5. Casper C, Sarapuk I, Pavlyshyn H. Regular and prolonged skin-to-skin contact improves short-term outcomes for very preterm infants: A dose-dependent intervention. Arch Pediatr. 2018;25(8):469-75.
6. Ramachandran S, Dutta S. Early developmental care interventions of preterm very low birth weight infants. Indian pediatrics. 2013;50(8):765-70.
7. Evereklian M, Posmontier B. The Impact of Kangaroo Care on Premature Infant Weight Gain. J Pediatr Nurs. 2017;34:e10-e6.
8. Bera A, Ghosh J, Singh AK, Hazra A, Mukherjee S, Mukherjee R. Effect of kangaroo mother care on growth and development of low birthweight babies up to 12 months of age: a controlled clinical trial. Acta Paediatr. 2014;103(6):643-50.
9. Bloch-Salisbury E, Zuzarte I, Indic P, Bednarek F, Paydarfar D. Kangaroo care: cardio-respiratory relationships between the infant and caregiver. Early Hum Dev. 2014;90(12):843-50.
10. Morelius E, Ortenstrand A, Theodorsson E, Frostell A. A randomised trial of continuous skin-to-skin contact after preterm birth and the effects on salivary cortisol, parental stress, depression, and breastfeeding. Early Hum Dev. 2015;91(1):63-70.
11. Butruille L, Blouin A, De Jonckheere J, Mur S, Margez T, Rakza T, et al. Impact of skin-to-skin contact on the autonomic nervous system in the preterm infant and his mother. Infant Behav Dev. 2017;49:83-6.
12. Cho ES, Kim SJ, Kwon MS, Cho H, Kim EH, Jun EM, et al. The Effects of Kangaroo Care in the Neonatal Intensive Care Unit on the Physiological Functions of Preterm Infants, Maternal-Infant Attachment, and Maternal Stress. J Pediatr Nurs. 2016;31(4):430-8.
13. Jefferies AL. Kangaroo care for the preterm infant and family. Paediatrics &

child health. 2012;17(3):141-6.
14. Campbell-Yeo ML, Disher TC, Benoit BL, Johnston CC. Understanding kangaroo care and its benefits to preterm infants. Pediatric Health Med Ther. 2015;6:15-32.
15. Barros FC, Bhutta ZA, Batra M, Hansen TN, Victora CG, Rubens CE. Global report on preterm birth and stillbirth (3 of 7): evidence for effectiveness of interventions. BMC pregnancy and childbirth. 2010;10 Suppl 1:S3.
16. Conde-Agudelo A, Diaz-Rossello JL. Kangaroo mother care to reduce morbidity and mortality in low birthweight infants. Cochrane Database Syst Rev. 2016(8):Cd002771.
17. Gnigler M, Ralser E, Karall D, Reiter G, Kiechl-Kohlendorfer U. Early sudden unexpected death in infancy (ESUDI)--three case reports and review of the literature. Acta Paediatr. 2013;102(5):e235-8.
18. Nakamura T, Sano Y. Two cases of infants who needed cardiopulmonary resuscitation during early skin-to-skin contact with mother. J Obstet Gynaecol Res. 2008;34(4 Pt 2):603-4.
19. Bohnhorst B, Heyne T, Peter CS, Poets CF. Skin-to-skin (kangaroo) care, respiratory control, and thermoregulation. The Journal of pediatrics. 2001;138(2):193-7.
20. Fischer CB, Sontheimer D, Scheffer F, Bauer J, Linderkamp O. Cardiorespiratory stability of premature boys and girls during kangaroo care. Early Hum Dev. 1998;52(2):145-53.
21. Mori R, Khanna R, Pledge D, Nakayama T. Meta-analysis of physiological effects of skin-to-skin contact for newborns and mothers. Pediatr Int. 2010;52(2):161-70.
22. Baley J. Skin-to-Skin Care for Term and Preterm Infants in the Neonatal ICU. Pediatrics. 2015;136(3):596-9.
23. Cooper L, Morrill A, Russell RB, Gooding JS, Miller L, Berns SD. Close to me: enhancing kangaroo care practice for NICU staff and parents. Advances in neonatal care : official journal of the National Association of Neonatal Nurses. 2014;14(6):410-23.
24. Vittner D, McGrath J, Robinson J, Lawhon G, Cusson R, Eisenfeld L, et al. Increase in Oxytocin From Skin-to-Skin Contact Enhances Development of Parent-Infant Relationship. Biological research for nursing. 2018;20(1):54-62.
25. O'Connor DT, Frigon RP, Sokoloff RL. Human chromogranin A. Purification and characterization from catecholamine storage vesicles of human pheochromocytoma. Hypertension (Dallas, Tex : 1979). 1984;6(1):2-12.

26. Smith AD, Winkler H. Purification and properties of an acidic protein from chromaffin granules of bovine adrenal medulla. The Biochemical journal. 1967;103(2):483-92.
27. Smith WJ, Kirshner N. A specific soluble protein from the catecholamine storage vesicles of bovine adrenal medulla. I. Purification and chemical characterization. Molecular pharmacology. 1967;3(1):52-62.
28. Nakane H, Asami O, Yamada Y, Harada T, Matsui N, Kanno T, et al. Salivary Chromogranin A as an index of psychosomatic stress response. Biomedical Research. 1998;19(6):401-6.
29. Den R, Toda M, Nagasawa S, Kitamura K, Morimoto K. Circadian rhythm of human salivary chromogranin A. Biomedical research (Tokyo, Japan). 2007;28(1):57-60.
30. Yamanaka Y, Motoshima H, Uchida K. Hypothalamic-pituitary-adrenal axis differentially responses to morning and evening psychological stress in healthy subjects. Neuropsychopharmacol Rep. 2019;39(1):41-7.
31. Ivars K, Nelson N, Theodorsson A, Theodorsson E, Strom JO, Morelius E. Development of Salivary Cortisol Circadian Rhythm and Reference Intervals in Full-Term Infants. PloS one. 2015;10(6):e0129502.
32. Morelius E, Theodorsson E, Nelson N. Salivary cortisol and mood and pain profiles during skin-to-skin care for an unselected group of mothers and infants in neonatal intensive care. Pediatrics. 2005;116(5):1105-13.
33. Lima AP, Beelen P, Bakker J. Use of a peripheral perfusion index derived from the pulse oximetry signal as a noninvasive indicator of perfusion. Critical care medicine. 2002;30(6):1210-3.
34. McGrath SP, Ryan KL, Wendelken SM, Rickards CA, Convertino VA. Pulse oximeter plethysmographic waveform changes in awake, spontaneously breathing, hypovolemic volunteers. Anesthesia and analgesia. 2011;112(2):368-74.
35. Partridge BL. Use of pulse oximetry as a noninvasive indicator of intravascular volume status. Journal of clinical monitoring. 1987;3(4):263-8.
36. Granelli A, Ostman-Smith I. Noninvasive peripheral perfusion index as a possible tool for screening for critical left heart obstruction. Acta Paediatr. 2007;96(10):1455-9.
37. Takahashi S, Kakiuchi S, Nanba Y, Tsukamoto K, Nakamura T, Ito Y. The perfusion index derived from a pulse oximeter for predicting low superior vena cava flow in very low birth weight infants. J Perinatol. 2010;30(4):265-9.
38. Piasek CZ, Van Bel F, Sola A. Perfusion index in newborn infants: a

noninvasive tool for neonatal monitoring. Acta Paediatr. 2014;103(5):468-73.
39. Van Laere D, O'Toole JM, Voeten M, McKiernan J, Boylan GB, Dempsey E. Decreased Variability and Low Values of Perfusion Index on Day One Are Associated with Adverse Outcome in Extremely Preterm Infants. The Journal of pediatrics. 2016;178:119-24.e1.
40. Cresi F, Pelle E, Calabrese R, Costa L, Farinasso D, Silvestro L. Perfusion index variations in clinically and hemodynamically stable preterm newborns in the first week of life. Italian journal of pediatrics. 2010;36:6.
41. Sheng Y, Zhu L. The crosstalk between autonomic nervous system and blood vessels. International journal of physiology, pathophysiology and pharmacology. 2018;10(1):17-28.
42. Gitau R, Modi N, Gianakoulopoulos X, Bond C, Glover V, Stevenson J. Acute effects of maternal skin-to-skin contact and massage on saliva cortisol in preterm babies. Journal of Reproductive and Infant Psychology. 2002;20(2):83-8.
43. Beijers R, Cillessen L, Zijlmans MA. An experimental study on mother-infant skin-to-skin contact in full-terms. Infant Behav Dev. 2016;43:58-65.

Table 1: Infants enrolled in the study (N=12)

	Average ± SD
gestational week	28.1 ± 2.6 weeks
birth weight	991.7 ± 347.6 grams
days before the initiation of oral feeding	1.5 ± 0.9 days
total minutes of previous SSC	887.5 ± 488.3 minutes
age (days, on the day of analysis)	56.3 ± 23.3 days
body weight (on the day of analysis)	1858.3 ± 178.2 grams
feed volume (on the day of analysis)	34.2 ± 3.9 ml/time

Table 2: Association between infant variables and post-SSC s-CgA/baseline s-CgA

		N	post-SSC s-CgA/baseline s-CgA (median, range)	P value
gender	girls	8	0.77 (0.61–0.97)	0.93
	boys	4	0.76 (0.54–2.58)	
respiratory support	+	6	0.95 (0.57–1.01)	0.63
	-	6	0.69 (0.60–0.78)	
sleep state	1	6	0.77 (0.64–0.91)	0.52
	2	6	0.82 (0.58–5.31)	
nutrition on the day of analysis	mother's milk	10	0.69 (0.58–0.91)	0.03
	mother's milk + formula milk	2	7.11 (6.93–7.30)	

FIGURE LEGENDS

Figure 1.

Analysis procedure before, during and after SSC.

Oxygen saturation (SpO_2), pulse rate and perfusion index were recorded before, during and after SSC. Saliva samples were collected from infants before initiating SSC and immediately after SSC to measure CgA and s-IgA. The SSC length varied among subjects and was at the discretion of the infant's mother.

Figure 2.

A. Comparison of the baseline salivary CgA between infants who were fed only their mother's milk before SSC on the day of examination (n=10) and those supplemented with formula milk in addition to their mother's milk before SSC (n=2; P=0.03). The horizontal bar in the histogram represents the median CgA value.

B. Comparison of the baseline salivary CgA and post SSC CgA in the infants who were fed only their mother's milk before SSC on the day of examination (n=10; P=0.009).

C. Baseline salivary CgA and post SSC CgA in the infants who were supplemented with formula milk in addition to their mother's milk before SSC on the day of

examination (n=2).

Figure 3

A. Perfusion index of the infants before, during and after SSC.

The perfusion index during SSC was significantly higher than that before or after SSC. The horizontal bar in the histogram represents the median value.

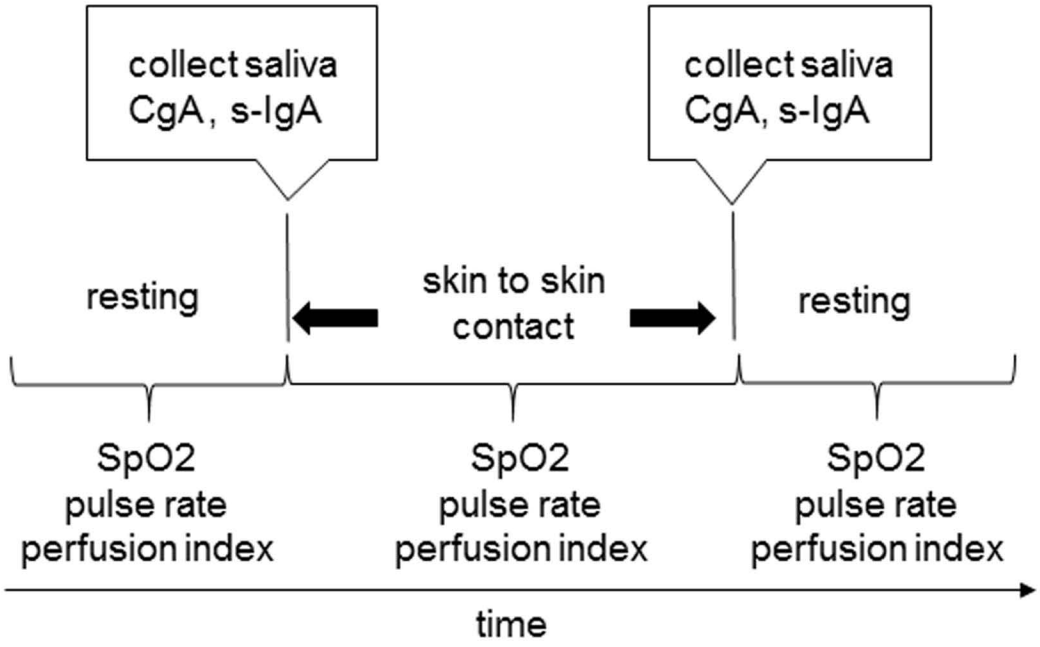
B. Oxygen saturation of the infants before, during and after SSC.

No significant change was found in oxygen saturation during or after SSC compared with that before SSC.

C. Pulse rate of the infants before, during and after SSC

No significant change was observed in the pulse rate during or after SSC compared with that before SSC.

Figure 1


collect saliva
CgA, s-IgA
collect saliva
CgA, s-IgA
resting
skin to skin
contact
resting
SpO2
pulse rate
perfusion index
SpO2
pulse rate
perfusion index
SpO2
pulse rate
perfusion index
time

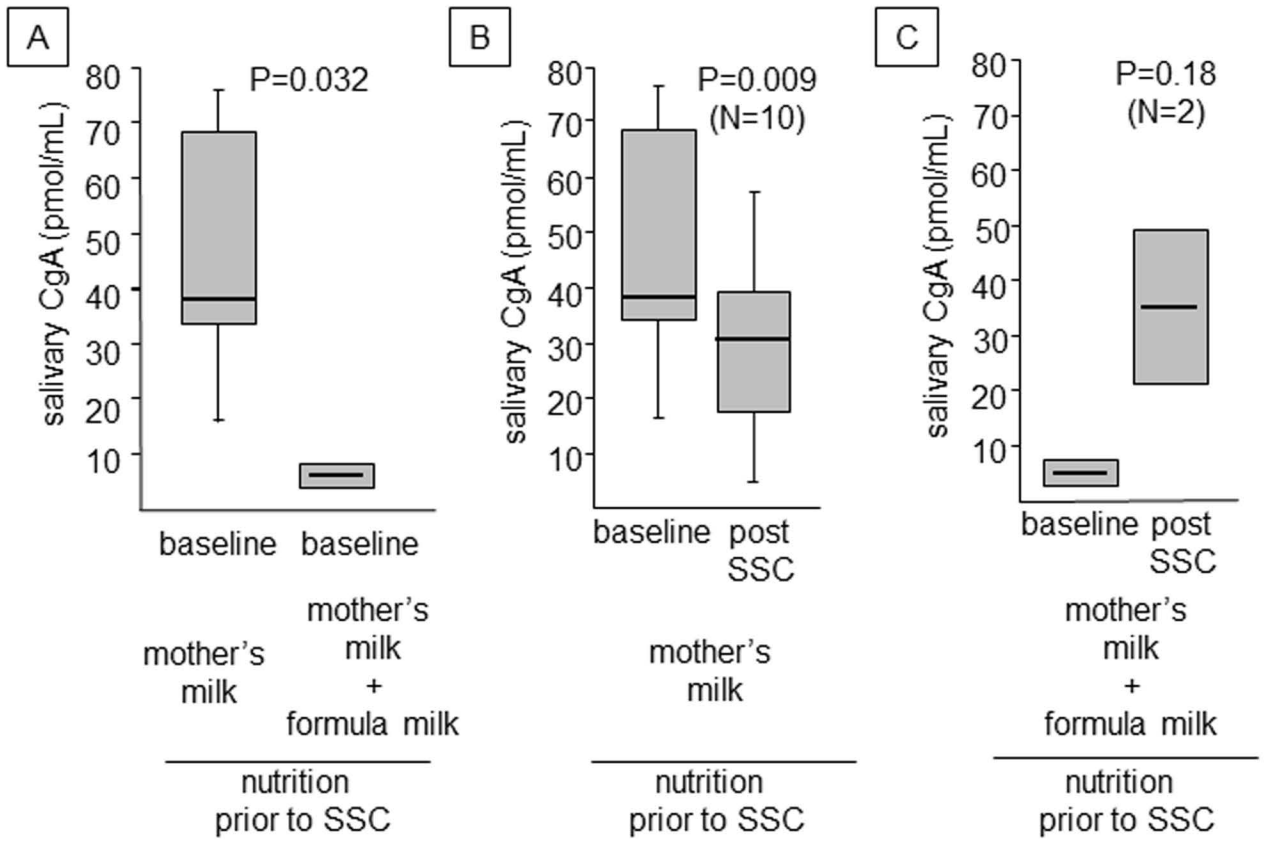
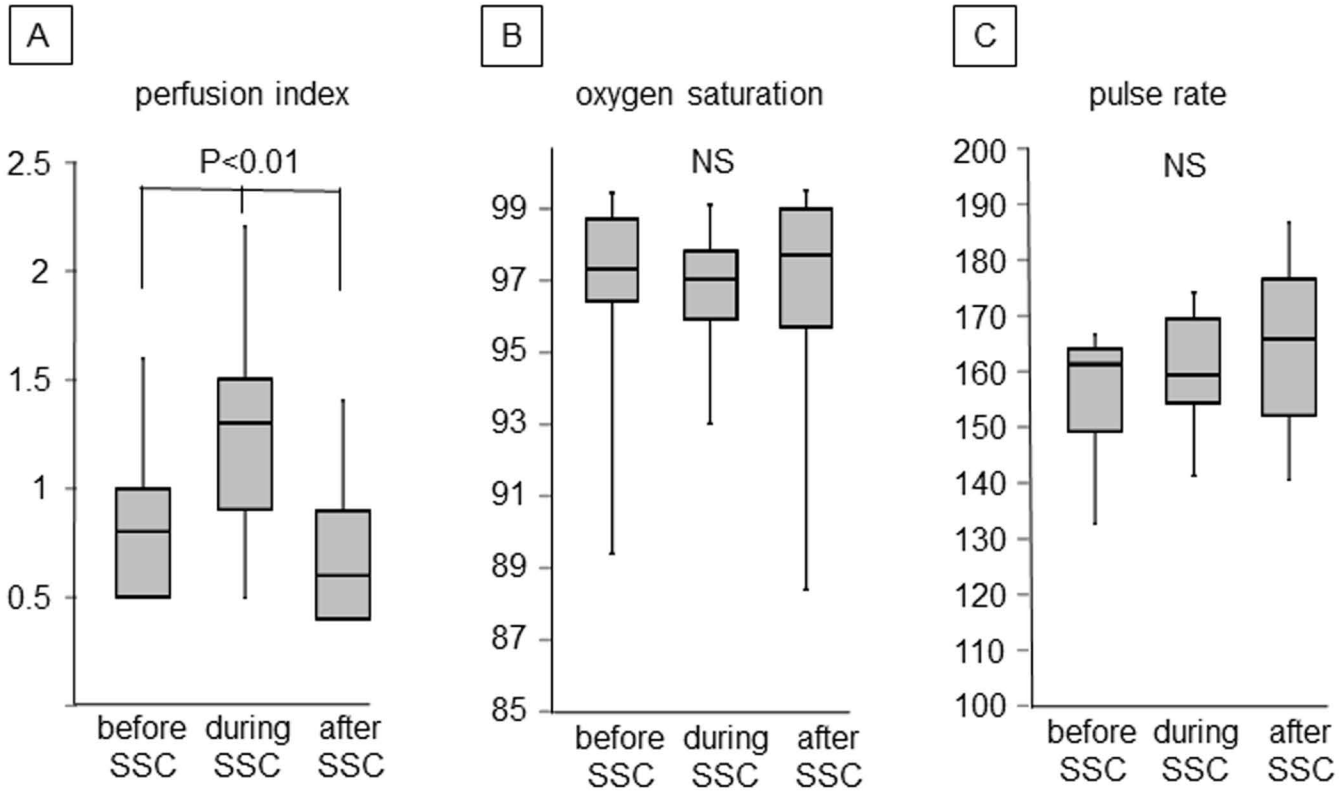

A
B
C
salivary CgA (pmol/mL)
80
70
60
50
40
30
20
10
P=0.032
P=0.009
(N=10)
P=0.18
(N=2)
baseline
baseline
post
SSC
mother's
milk
mother's
milk
+
formula milk
nutrition
prior to SSC

Figure 3


A
perfusion index
P<0.01
2.5
2
1.5
1
0.5
before SSC
during SSC
after SSC
B
oxygen saturation
NS
99
97
95
93
91
89
87
85
before SSC
during SSC
after SSC
C
pulse rate
NS
200
190
180
170
160
150
140
130
120
110
100
before SSC
during SSC
after SSC