

Infant Massage and Salivary Cortisol: A Literature Review

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Infant Massage

Theory/Importance/Background

Title: Elevated maternal anxiety in the NICU predicts worse fine motor outcome in VLBW infants

Citation: Greene MM, Rossman B, Meier P, Patra K. Elevated maternal anxiety in the NICU predicts worse fine motor outcome in VLBW infants. *Early Hum Dev.* 2018;116:33-39. doi:10.1016/j.earlhumdev.2017.10.008

Methods: 69 mothers and their very low birth weight infants participated in a mixed methods study (quantitative findings discussed in this article). Infant and maternal data was extracted from the medical records and mothers completed self-report distress questionnaires shortly after birth and shortly prior to discharge. Infants were assessed at a NICU Follow Up Clinic to determine post-NICU development at 20 months corrected age.

Results: Elevated maternal anxiety at the first data collection point (2-4 weeks after birth) was associated with decreased fine motor scores on the Bayley at 20 months corrected age, while elevated depression was not associated with a decrease in any scores on the Bayley at 20 months corrected age. Researchers hypothesized that this may be due to decreased maternal attachment that has been shown to result from increased maternal anxiety in preterm infants.

Title: Mothers and fathers in NICU: the impact of preterm birth on parental distress

Citation: Ionio C, Colombo C, Brazzoduro V, et al. Mothers and fathers in NICU: the impact of preterm birth on parental distress. *Eur J Psychol.* 2016;12(4):604-621. doi:10.5964/ejop.v12i4.1093

Methods: 21 couples of preterm infants and 29 couples of term infants participated in this longitudinal study. Parents filled out questionnaires assessing trauma-related symptoms, parents' affective states, social support levels, post partum bonding, and parent stress scale (NICU specific, so only administered to preterm infant parents). These questionnaires were filled out 1-2 weeks after their child's birth.

Results: Higher levels of tension-anxiety, depression, anger-hostility, and fatigue were all found in preterm infant mothers compared to term infant mothers. Fathers of preterm infants were found to have lower levels of vigor-activity and higher levels of hostility-anger than fathers of term infants. Comparing mothers and fathers of preterm infants, mothers were found to have higher levels of intrusive feelings, hyperactivity, general stress, altered parental role stress, and stress related to their baby's appearance.

Title: Factors associated with the quality of life of mothers of preterm infants with very low birth weight: a 3-year follow-up study

Citation: Moura MRS, Araújo CGA, Prado MM, et al. Factors associated with the quality of life of mothers of preterm infants with very low birth weight: a 3-year follow-up study. *Qual Life Res.* 2017;26(5):1349-1360. doi:10.1007/s11136-016-1456-6

Methods: All mothers participating in the study were interviewed at maternal discharge, 6 months, 12 months, 24 months, and 36 months after delivery. They completed questionnaires on both maternal and infant sociodemographic and medical information. Mothers also completed a depression and quality of life screening questionnaire, and infants completed a

developmental screen at the last 4 time points. 75 mothers participated at the initial time point, although this number was down to 32 at the 36 month follow up.

Results: Higher levels of depressed mood (not necessarily depression) were found at discharge. For infants, more infants were classified as having abnormal developmental scores at 24 months than 6 months. Mothers reported having improved feelings of overall wellbeing 6 and 12 months after discharge. Mothers with increased depressive symptoms have been found to interact less with their children, which could have negative implications for development.

Title: Outcomes for extremely premature infants

Citation: Glass HC, Costarino AT, Stayer SA, Brett CM, Cladis F, Davis PJ. Outcomes for extremely premature infants. *Anesth Analg.* 2015;120(6):1337-1351.
doi:10.1213/ANE.0000000000000705

Methods: This was a narrative review synthesizing literature about short- and long-term outcomes for extremely preterm infants.

Results: Despite medical advances that have significantly improved outcomes, premature birth remains a substantial contributor to infant mortality, in addition to leading to a host of medical complications for many of those who do survive. These outcomes can be even more severe for extremely preterm infants.

Title: Recommendations for involving the family in developmental care of the NICU baby

Citation: Craig JW, Glick C, Phillips R, Hall SL, Smith J, Browne J. Recommendations for involving the family in developmental care of the NICU baby. *J Perinatol.* 2015;35 Suppl 1:S5-8.
doi:10.1038/jp.2015.142

Methods: This was a review performed based on existing literature.

Results: They recommended increasing the role of the family during an infant's time in the NICU to minimize the negative effects of a NICU stay on parent-infant attachment. An impaired attachment can lead to impairments in social, emotional, behavioral, and cognitive functioning in preterm infants. Incorporating the family into care has been shown to decrease maternal stress scores, in addition to improving outcomes for the infants. Some of the recommendations to increase family-centered care was related to having the parents provide increased hands-on care to promote skin-to-skin contact and infant touch.

Preterm birth can be a stressful experience for parents, and research indicates that parents can experience increased levels of depression, stress, and anxiety. These indicators have been shown in longitudinal studies to also impact infant development, which is thought to be due to decreased parent-infant synchrony and attachment. Current models are exploring ways to involve the family more in care during the NICU to help improve maternal outcomes and, consequently, infant outcomes.

Feasibility/Acceptability

Title: A Maternal-Administered Multimodal Neonatal Bundle in Hospitalized Very Preterm Infants: A Pilot Study

Citation: Letzkus L, Alonzo C, Connaughton E, Kelly N, Zanelli S. A Maternal-Administered Multimodal Neonatal Bundle in Hospitalized Very Preterm Infants: A Pilot Study. *Adv Neonatal Care*. 2021;21(2):E35-E42. doi:10.1097/ANC.0000000000000786

Methods: 13 mothers of preterm infants in a level IV NICU were taught a bundle of 5 interventions (vocal soothing, scent exchange, comforting touch, kangaroo care, and infant massage). Massage was instructed to be performed for 15 minutes two times a day. Feasibility (performing the intervention 5 days per week) and acceptability were assessed. Additionally, mothers completed self-report questionnaires assessing stress, anxiety, and depression.

Results: Vocal soothing, scent exchange, and comforting touch all met the desired feasibility threshold; however, kangaroo care was performed on average 48.3% of the time and infant massage was performed on average 45.2% of the time. Infant massage lasted for around 10 minutes rather than the desired target of 15 minutes. There was a significant decrease in maternal stress after completing the intervention bundle. There was a nonsignificant decline in maternal anxiety and a significant decline in depressive symptoms. Researchers postulated that additional support may be needed in order to improve rates of adherence to kangaroo care and massage protocols.

Title: Parent preferences for motor development education in the neonatal intensive care unit

Citation: Dusing SC, Murray T, Stern M. Parent preferences for motor development education in the neonatal intensive care unit. *Pediatr Phys Ther*. 2008;20(4):363-368. doi:10.1097/PEP.0b013e31818add5d

Methods: This was a series of focus groups designed to assess modes of education and clarity of education materials for parent education in the NICU.

Results: A majority of parents reported that they preferred observing how to interact with their child at the same time as receiving verbal information. Written information was deemed the least helpful, and most parents said they would prefer a combination of demonstration/verbal/written. Participants in the second focus group demonstrated an increased knowledge of how to interact and play with their infants, emphasizing the need for education with these parents in order to facilitate optimal parent-infant attachment.

Title: Effects of instruction on parent competency during infant handling in a neonatal intensive care unit

Citation: Byrne EM, Sweeney JK, Schwartz N, Umphred D, Constantinou J. Effects of instruction on parent competency during infant handling in a neonatal intensive care unit. *Pediatr Phys Ther*. 2019;31(1):43-49. doi:10.1097/PEP.0000000000000557

Methods: Participants were randomized into 3 groups (direct instruction and demonstration, video instruction and demonstration, written-pictorial instruction) to assess the effectiveness of different education methods. Miller's pyramid of competency was used to assess parent knowledge and infant illness information/demographic information was also collected. Parents

were tested on their ability to complete 3 handling tasks: moving the infant into sidelying, facilitation of free kicking/guided constrained kicking, and supported sitting.

Results: Direct and video instruction were both found to lead to significantly higher levels of parent competency for the first two activities (trend for supported sitting but did not reach significance). They also found a difference based on level of parent education; parents with no college education scored highest when video instruction was used, but parents with a college education scored lowest when video instruction was used.

Title: Maternal satisfaction with administering infant interventions in the neonatal intensive care unit

Citation: Holditch-Davis D, White-Traut R, Levy J, Williams KL, Ryan D, Vonderheid S. Maternal satisfaction with administering infant interventions in the neonatal intensive care unit. *J Obstet Gynecol Neonatal Nurs*. 2013;42(6):641-654. doi:10.1111/1552-6909.12255

Methods: 208 mothers of preterm infants were randomized into 3 groups (ATVV, kangaroo care, control) to assess and compare satisfaction with/between the interventions across 4 hospitals. Participants completed a satisfaction survey at discharge and at 2 months corrected age. Additionally, mothers were not prevented from engaging in other interventions; however, they only received instruction on the intervention for their assigned group from the study nurse. Study nurses maintained contact at least weekly during hospitalization and monthly after discharge. Mothers completed other questionnaires assessing maternal anxiety, depressive symptoms, post-traumatic stress symptoms, worry about child health, demographics, and infant illness characteristics.

Results: In all 3 groups, mothers were satisfied with their intervention and the study nurse's helpfulness. However, mothers in the ATVV group had significantly higher scores on the statement "learn new ways to stimulate and teach my infant," while mothers in the kangaroo care group had significantly higher scores for the statement "feel like I was helping my baby while in the hospital". This change persisted at 2 months for mothers in the ATVV group. Mothers in both intervention groups reported that they felt more like a mother during hospitalization than mothers in the control group.

Maternal satisfaction and improved health outcomes have been noted with massage-based interventions. However, more research on how to increase adherence to desired interventions is still needed. Parents report preferring multiple modes of instruction on how to interact with their infants, with direct observation of a physical therapist tending to be the most popular mode of instruction. However, it is also important to consider parent education level when designing intervention instructions.

Cortisol Responses

Title: Salivary cortisol and behavioral state responses of healthy newborn infants to tactile-only and multisensory interventions

Citation: White-Traut RC, Schwertz D, McFarlin B, Kogan J. Salivary cortisol and behavioral state responses of healthy newborn infants to tactile-only and multisensory interventions. *J Obstet Gynecol Neonatal Nurs*. 2009;38(1):22-34. doi:10.1111/j.1552-6909.2008.00307.x

Methods: 60 healthy newborns received either a 15-minute ATVV intervention, 15 minutes of only the tactile component of the ATVV intervention, or routine hospital care. Salivary cortisol levels were collected before the intervention, upon completion of the intervention, and 10 minutes after completion of the intervention. Additionally, infant behavioral state was classified (by a blinded, independent assessor) before the session and at the end of every minute during the intervention.

Results: Due to issues collecting sufficient levels of salivary cortisol, only 40 infants were included in the final analysis. Both the control group and tactile-only group had increases in cortisol levels from pre- to post-intervention. Infants in the ATVV group had a decline in cortisol from pre- to post- to 10 minutes post-intervention. There was a significant difference in cortisol levels between the ATVV group and the other two groups. All three groups had a “sleep” state as the predominant infant behavioral state throughout the intervention.

Title: The Stockholm Neonatal Family-Centered Care Study: effects on salivary cortisol in infants and their mothers

Citation: Mörelius E, Broström EB, Westrup B, Sarman I, Örténstrand A. The Stockholm Neonatal Family-Centered Care Study: effects on salivary cortisol in infants and their mothers. *Early Hum Dev*. 2012;88(7):575-581. doi:10.1016/j.earlhumdev.2011.12.033

Methods: Infants in two NICUs in Stockholm were placed into either a family care unit or a standard care unit. In the family-centered units, one parent was expected to be present at all times until discharge. However, parents in the standard care units were not able to stay overnight as easily. 289 infants and their families participated in this study. Salivary cortisol was collected before and 30 minutes after mothers changed their infant’s diaper.

Results: There was no difference in salivary cortisol levels among infants or mothers in the two groups. In the family-centered care group, significant correlations were found between mother and infant cortisol levels at both time points; these correlations were not found for the standard care group. This could indicate increased harmony/bonding between mother-infant dyads in a family-centered care approach.

ATVV interventions have been found to decrease cortisol levels (as compared to other interventions) in healthy, term infants, although this has not been assessed in premature infants. Additionally, there is research that increased interaction between mother-infant dyads may lead to increased cohesiveness with levels of and changes in salivary cortisol levels.

Maternal Anxiety/Distress

Title: Early postnatal maternal trait anxiety is associated with the behavioral outcomes of children born preterm <33 weeks

Citation: Kleine I, Falconer S, Roth S, et al. Early postnatal maternal trait anxiety is associated with the behavioural outcomes of children born preterm <33 weeks. *J Psychiatr Res.* 2020;131:160-168. doi:10.1016/j.jpsychires.2020.09.010

Methods: 140 preterm infants were assessed longitudinally at 2 and 4-6 years of age. Clinical and sociodemographic information was collected upon enrollment for infants, neurodevelopmental assessment was completed at age 2 years, and behavioral/cognitive assessments were completed between 4-6 years of age. Maternal demographics were also collected upon enrollment. Maternal trait anxiety was assessed for all mothers in the study at the child's term-equivalent age, 14 days corrected age, 12 months corrected age, and 22 months corrected age.

Results: Increased levels of behavioral problems were associated with increased levels of maternal trait anxiety at term-equivalent age specifically and when averaged across 4 time points. Greater deficits in social behavior were also associated with increased maternal trait anxiety. Even at term-corrected age, increased anxiety in mothers in the first two years of life led to poorer behavioral outcomes between ages 4-6 years.

Title: Depression and anxiety symptoms of mothers of preterm infants are decreased at 4 months corrected age with Family Nurture Intervention in the NICU

Citation: Welch MG, Halperin MS, Austin J, et al. Depression and anxiety symptoms of mothers of preterm infants are decreased at 4 months corrected age with Family Nurture Intervention in the NICU. *Arch Womens Ment Health.* 2016;19(1):51-61. doi:10.1007/s00737-015-0502-7

Methods: 115 families with very preterm infants participated in this study and were assigned to the Family Nurture Intervention or standard care groups. The family nurture intervention consisted of scent cloth exchange, calming touch, and holding. Families in standard care could engage in interventions of their choice but received no education from the study nurses. Mothers completed self-report measures at enrollment, near term age, and at 4 months corrected age to assess psychological state (depression, state/trait anxiety, and motivation systems).

Results: At 4 months, there were significantly lower levels of depression and state anxiety among mothers who completed the family nurture intervention. There was also a trend for decreased levels of state anxiety in the family nurture intervention group at the near-term evaluation time point.

Title: Maternally administered interventions for preterm infants in the NICU: effects on maternal psychological distress and mother-infant relationship

Citation: Holditch-Davis D, White-Traut RC, Levy JA, O'Shea TM, Geraldo V, David RJ. Maternally administered interventions for preterm infants in the NICU: effects on maternal psychological distress and mother-infant relationship. *Infant Behav Dev.* 2014;37(4):695-710. doi:10.1016/j.infbeh.2014.08.005

Methods: 240 mother-infant dyads were assigned to either an ATVV intervention, kangaroo care intervention, or attention control intervention. The ATVV intervention took place over 15 minutes and involved voice, moderate stroking/massage, eye-to-eye contact, and horizontal rocking. Kangaroo care was performed for at least 15 minutes, although mothers were told they could continue for longer if desired. The control group received education on how to identify equipment needed to care for preterm infants. All mothers were taught their interventions and asked to perform them until the infant was 2 months corrected age. Mothers used a diary to track their completion of interventions and any other interactions with their infants. Infant sleep-wake responses to the intervention were recorded, and mothers completed self-report measures to assess depressive symptoms, state anxiety, post-traumatic stress symptoms, and parenting stress. The mother-infant relationship was also assessed based on video interactions of mother-infant dyads.

Results: Mothers completing massage interventions (including ATVV) had a significantly faster decline in depressive symptoms than mothers not completing an intervention (difference not noted for mothers who performed kangaroo care). Parenting stress was significantly lower for parents engaging in either intervention as compared to controls. Mothers in the kangaroo care group had a faster decline in worry about their infants (although non-significant). Better home environments were also noted for mothers who performed massage-based interventions.

Title: Effects of infant massage on state anxiety in mother of preterm infants prior to hospital discharge

Citation: Afand N, Keshavarz M, Fatemi NS, Montazeri A. Effects of infant massage on state anxiety in mothers of preterm infants prior to hospital discharge. *J Clin Nurs*. 2017;26(13-14):1887-1892. doi:10.1111/jocn.13498

Methods: Mothers completed self-report questionnaires to collect demographic information. Baseline state anxiety levels were assessed and then mothers in the experimental group participated in an 8-minute infant massage intervention the day prior to and the day of their infant's discharge. State anxiety levels were again assessed immediately after the intervention and 15 to 30 minutes after the intervention on the day of discharge for both groups.

Results: Mothers in the experimental group had significantly lower levels of anxiety than mothers in the control group, although both groups had significant decreases in cortisol levels (possibly related to infant's impending discharge).

Title: Parental engagement and early interactions with preterm infants reduce risk of late postpartum depression

Citation: Xie J, Zhu L, Zhu T, et al. Parental engagement and early interactions with preterm infants reduce risk of late postpartum depression. *J Nerv Ment Dis*. 2019;207(5):360-364. doi:10.1097/NMD.0000000000000971

Methods: 151 mother-preterm infant dyads either received an early parent interaction intervention or standard care. The intervention consisted of 9 sessions where parents were instructed in twice daily massage therapy and visual attention stimulation. Demographic and neonatal variables were collected upon enrollment. Mothers completed self-report questionnaires at discharge related to knowledge of child development/adjustment and at one

year of age related to adjustment. Mothers were also observed playing with their infant for 10 minutes, and infant development behavior was assessed at one year.

Results: There was no change in infant development, early maternal adjustment, or the mother-infant relationship. However, there were significantly fewer mothers in the intervention group who were identified as being “at risk” for depression at the 12 month follow up than in the control group, indicating a reduced risk for postpartum depression.

Title: Mothers are not fathers: differences between parents in the reduction of stress levels after a parental intervention in a NICU

Citation: Matricardi S, Agostino R, Fedeli C, Montirosso R. Mothers are not fathers: differences between parents in the reduction of stress levels after a parental intervention in a NICU. *Acta Paediatr.* 2013;102(1):8-14. doi:10.1111/apa.12058

Methods: All parents and infants received standard care in the NICU, which included information on kangaroo care and meetings with psychologists and physical therapists. Parents in the intervention group also had 8 additional sessions with a physical therapist which included observation and completion of infant massage by both parents. Parents provided sociodemographic information in addition to completing self-report measures of parental stress a week after their infant was admitted to the NICU and upon discharge. The infant’s clinical status was also calculated at birth and recorded.

Results: Mothers reported higher stress than fathers related to the sights/sounds of the NICU, infant appearance, and parental role alteration. All parents had higher stress levels at the time of infant admission than at discharge. There was a significant increase in stress related to infant appearance for parents in the control group, while there was a significant decrease for parents in the intervention group. There was a significant increase in stress associated with sights/sounds for parents in the control group. Parental role alteration stress scores decreased significantly for mothers but did not for fathers. Mothers in the intervention group also reported less role-related stress at infant discharge, but fathers did not. This indicates that massage-based interventions may have different impacts in mothers and fathers.

Title: Mothers’ depressed mood and anxiety levels are reduced after massaging their preterm infants

Citation: Feijó L, Hernandez-Reif M, Field T, Burns W, Valley-Gray S, Simco E. Mothers’ depressed mood and anxiety levels are reduced after massaging their preterm infants. *Infant Behav Dev.* 2006;29(3):476-480. doi:10.1016/j.infbeh.2006.02.003

Methods: 40 mother-preterm infant dyads were included, and mothers either performed 8 minutes of infant massage or observed 8 minutes of infant massage being performed on their infant. Mothers completed self-report measures on state anxiety and depression pre- and post-massage. They also completed the Infant Massage Questionnaire.

Results: There was a significant decrease in state anxiety levels for mothers in the intervention group but not the control group, and there was a significant decrease in depressive mood for mothers in both groups.

Research has shown that increased maternal anxiety in the first two years of life can lead to increased behavioral issues and decreased social behavior up to 6 years of age (no data

collected past this time point), indicating the need for interventions to reduce maternal anxiety as early as possible. Various treatment protocols involving infant massage have found decreased levels of anxiety, parenting stress, and depressive symptoms in mothers who participated compared to mothers in control groups. They have also indicated a better home environment for those completing infant massage. Additionally, one study investigated gender differences and found that while massage did decrease some levels of stress for both mothers and fathers, others were unaffected in fathers while decreased in mothers (parental role alteration and role-related stress at discharge). This indicates that massage interventions may have different impact on mothers versus fathers, although this needs further investigation.

Maternal and Infant Outcomes

Title: Enhancing sensory experiences for very preterm infants in the NICU: an integrative review

Citation: Pineda R, Guth R, Herring A, Reynolds L, Oberle S, Smith J. Enhancing sensory experiences for very preterm infants in the NICU: an integrative review. *J Perinatol.* 2017;37(4):323-332. doi:10.1038/jp.2016.179

Methods: This article was an integrative review assessing the use of sensory-based interventions to improve both parent and infant outcomes for very preterm infants in NICUs.

Results: They found evidence supporting improved mother-infant interaction after infant massage, improved mental/cognitive development after kangaroo care, improved mood in parents after kangaroo care, improved mother-infant interaction after kangaroo care (although some literature did not support these findings related to kangaroo care). They found improved developmental outcomes, improved tolerance for handling, and improved feeding for infants who received ATVV intervention (however not all literature supported this). They also found support for maternal outcomes discussed above. Massage interventions were also found to decrease maternal stress and improved mental development for infants at 2 years (also some mixed evidence). There were also reviews of auditory, visual, and gustatory/olfactory interventions.

Title: Parental psychological well-being and behavioral outcome of very low birth weight infants at 2 years

Citation: Huhtala M, Korja R, Lehtonen L, et al. Parental psychological well-being and behavioral outcome of very low birth weight infants at 3 years. *Pediatrics.* 2012;129(4):e937-44. doi:10.1542/peds.2011-2411

Methods: 140 very low birth weight infants and their parents participated in this study. Infant medical data was collected from the medical records, and parents self-reported background data. Parents completed self-report measures on depressive symptoms, parenting stress, and parental sense of coherence upon enrollment. At 2 years corrected age, children were assessed using the Bayley Scales of Infant Development, and at 3 years of age, parents completed a self-report assessment of their child's behavioral patterns.

Results: Decreased parental psychological wellbeing was associated with increased behavioral problems in children at 3 years of age. Maternal and paternal levels of depression, parenting stress, and sense of coherence were all found to be significant predictors of internalizing and externalizing behavioral problems in their child (except with respect to paternal depressive symptoms and child externalizing behavior problems).

One review found improved mother-infant interaction, decreased maternal stress, and improved infant mental/cognitive development for mother-infant dyads participating in infant massage or kangaroo care. Additionally, they found improved tolerance for handling and improved feeding for infants receiving ATVV. Further, research indicated that decreased parental psychological wellbeing was associated with increased internalizing behavior problems in children at 3 years of age, and decreased maternal psychological wellbeing was associated with increased externalizing behavior problems in children at 3 years of age.

Infant Outcomes

Title: Review and critical analysis of massage studies for term and preterm infants

Citation: Juneau AL, Aita M, Héon M. Review and critical analysis of massage studies for term and preterm infants. *Neonatal Netw.* 2015;34(3):165-177. doi:10.1891/0730-0832.34.3.165

Methods: This was a systematic review of the literature assessing infant outcomes after receiving massage (only massage not including other sensory components).

Results: With respect to preterm infants, improved weight gain was consistently found after massage interventions. In addition, infants had decreased pain responses when undergoing a painful procedure after receiving massage. Infants were also found to have improved social engagement at 3 months old and significantly improved Bayley scores at 12 months.

Title: Effectiveness of a modified Mother-Infant Transaction Program on outcomes for preterm infants from 3 to 24 months of age

Citation: Newnham CA, Milgrom J, Skouteris H. Effectiveness of a modified Mother-Infant Transaction Program on outcomes for preterm infants from 3 to 24 months of age. *Infant Behav Dev.* 2009;32(1):17-26. doi:10.1016/j.infbeh.2008.09.004

Methods: 63 mothers participated in this study and either received the intervention or control. In the intervention, mothers had seven hospital sessions over two weeks, in addition to a home visit one month after discharge and a hospital visit 3 months after discharge. Parents in the intervention group received the standard Mother-Infant Transaction Program intervention (education on recognizing different infant behavioral states and how to respond), in addition to education on kangaroo care, massage, and infant bath. Mothers completed self-report measures of depression at enrollment and 3- and 6-months corrected age. At enrollment, socioeconomic status and birth/medical information of the infant was also collected. Mothers completed an assessment of infant temperament and parenting stress at 3- and 6-months corrected age. A synchrony scale was completed by researchers after review of mother-infant interactions at 3- and 6-months corrected age. Parents also completed a self-report questionnaire of child development at 2 years of age.

Results: No group differences were found with respect to maternal depression. Mothers in the intervention group were found to be significantly more responsive at 3 months corrected age and their infants were found to be significantly more attentive at 3 and 6 months. Additionally, there was improved mother-infant synchrony at 3 months and mutual attention at 6 months. Mothers in the intervention group also reported a calmer temperament in their infant at 3 months than mothers in the control group. Mothers in the control group also had significantly higher levels of stress at 3 months corrected age than intervention group mothers.

Title: Effect of multisensory stimulation on neuromotor development in preterm infants

Citation: Kanagasabai PS, Mohan D, Lewis LE, Kamath A, Rao BK. Effect of multisensory stimulation on neuromotor development in preterm infants. *Indian J Pediatr.* 2013;80(6):460-464. doi:10.1007/s12098-012-0945-z

Methods: Infants in control group (n = 25) received NICU standard care (includes kangaroo care), while infants in the intervention group (n = 25) received NICU standard care in addition to ATVV intervention 12 minutes per day, 5 days per week. Baseline data for both groups was

obtained from the medical records. Physiological stress signs were monitored during the intervention. Neuromotor development was assessed at term age for infants in both groups.

Results: Infants in the intervention group had significantly higher scores on the neuromotor development assessment as a whole, in addition to significantly higher scores on two individual items (heel to ear popliteal angle and weight bearing in standing).

Title: Developmental Patterns of Physiological Response to a Multisensory Intervention in Extremely Premature and High-Risk Infants

Citation: White-Traut RC, Nelson MN, Silvestri JM, et al. Developmental Patterns of Physiological Response to a Multisensory Intervention in Extremely Premature and High-Risk Infants. *JOGNN*. 2004;33(2):266-275.

Methods: 37 premature infants participated in this study and received either current standard of care (control) or current standard of care and 15 minutes of ATVV intervention (experimental). Heart rate, respiratory rate, and oxygen saturation were measured throughout the intervention time frame.

Results: Infants in the intervention group had significantly higher heart rates and oxygen saturation rates during and immediately after the intervention, although these differences were not considered clinically significant.

Title: Effect of auditory, tactile, visual, and vestibular intervention on length of stay, alertness, and feeding progression in preterm infants

Citation: White-Traut RC, Nelson MN, Silvestri JM, et al. Effect of auditory, tactile, visual, and vestibular intervention on length of stay, alertness, and feeding progression in preterm infants. *Dev Med Child Neurol*. 2002;44:91-97.

Methods: 37 premature infants participated in this study and received either current standard of care (control) or current standard of care and 15 minutes of ATVV intervention 2 times per day 5 days per week (experimental). Data was collected related to infants' alert/sleep states over 1-week periods and feeding progression (number of days to complete nipple feeding).

Results: There were no group differences in behavioral state at enrollment, but quiet sleep significantly increased over the first week for the intervention group and then later decreased over time. The intervention group also had significantly more alertness at the beginning of the 15-minute intervention window, which was maintained across all time intervals.

Research has found a number of improved infant outcomes, such as improved weight gain, decreased pain response, improved social engagement, improved overall development, increased responsiveness/attentiveness/alertness, improved mother-infant synchrony and mutual attentiveness, and increased presence of a calm temperament, after receiving infant massage from their mothers. Additionally, one study found no clinically significant change in physiological states during infant massage, indicating safety as an intervention measure in preterm infants.

Mother-Infant Interaction/Attachment

Title: Increase in Oxytocin from Skin-to-Skin Contact Enhances Development of Parent-Infant Relationship

Citation: Vittner D, McGrath J, Robinson J, et al. Increase in Oxytocin From Skin-to-Skin Contact Enhances Development of Parent-Infant Relationship. *Biol Res Nurs*. 2018;20(1):54-62. doi:10.1177/1099800417735633

Methods: Mother-father-infant triads participated in skin-to-skin contact with infants receiving skin-to-skin contact with one parent on the first day of the intervention and the other parent on the second day of the intervention. Parent and infant oxytocin and cortisol samples were taken before, during, and after the intervention. Parents also self-reported anxiety levels, and examiners determined levels of parent-infant synchrony and responsiveness from observation of interactions. Mothers or fathers engaged in 60 minutes of skin-to-skin contact with their infant during the intervention time frame.

Results: Mothers had significant increases in oxytocin from before to during the intervention, followed by a significant drop post-intervention (infants had the same pattern with maternal skin-to-skin contact). This pattern was also seen for fathers and infants during paternal skin-to-skin contact. Infants also demonstrated significantly lower cortisol levels during the intervention with either parent than before or after the intervention (mothers and fathers had the same trend, although the differences were not significant). Maternal and paternal anxiety levels were significantly lower during the intervention than before or after the intervention. Parent-infant dyads with increased oxytocin levels during skin-to-skin contact were found to have increased synchrony/responsiveness.

Title: Mother and child behavior in very preterm and term dyads at 6 and 8 years

Citation: Jaekel J, Wolke D, Chernova J. Mother and child behaviour in very preterm and term dyads at 6 and 8 years. *Dev Med Child Neurol*. 2012;54(8):716-723. doi:10.1111/j.1469-8749.2012.04323.x

Methods: 267 mother-preterm infant dyads participated in this study, with 298 mother-infant dyads serving as a comparison group. Pregnancy and neonatal data were collected from the medical records and social information was collected from discussion with mothers shortly after birth. At 6 years old, cognitive assessments were performed on all children, and at 6 and 8 years, mothers were observed completing an Etch a Sketch play simulation with their children.

Results: Preterm infants were found to be less socially interactive and less task persistent at ages 6 and 8 when compared to term peers. Additionally, mothers of premature infants were less sensitive and more verbally controlling at both 6 and 8 years, and decreased harmony was noted at 6 years. Maternal sensitivity at 6 years of age was found to be a significant predictor of task persistence at 8 years of age, and this effect was found to be larger for children with lower IQ scores.

Title: Mother-infant interaction improves with a developmental intervention for mother-preterm infant dyads

Citation: White-Traut R, Norr KF, Fabiyi C, Rankin KM, Li Z, Liu L. Mother-infant interaction improves with a developmental intervention for mother-preterm infant dyads. *Infant Behav Dev.* 2013;36(4):694-706. doi:10.1016/j.infbeh.2013.07.004

Methods: 142 mother-infant dyads were assigned to either a parent education control group or H-HOPE intervention group. The intervention consisted of a 15-minute ATVV intervention two times per day, in addition to education/social support for mothers (including 2 home visits and 2 phone calls after discharge) related to feeding/weighting the infant and maternal stress/support. Parents in the control group received the same amount of interaction with study staff but received basic information about premature infant care and car safety. Mother-infant interaction was assessed by study researchers during a feeding and play at 6 weeks corrected age. Infant medical data was collected from the EMR, and maternal demographic information was collected during an interview with the parent. Mothers completed a self-report score for depression and trait anxiety.

Results: During feeding, intervention dyads were found to have increased positive interactions, although this finding was not statistically significant. Only the infant clarity of cues subscale was found to be significantly higher in the intervention group. During play, there were fewer dyads in the intervention group that demonstrated low levels of responsiveness (significant).

Research has indicated that premature infants are less socially interactive/task persistent in early-middle childhood and tend to have mothers who are less sensitive and more verbally controlling, in addition to having decreased harmony in the mother-child relationship. This indicates the need for interventions that help improve child social behavior and the mother-child relationship. One study found that skin-to-skin contact with either parent led to significant increases in oxytocin levels and decreases in cortisol levels (significant for infant only) in mothers, fathers, and infants; the increase in oxytocin levels was associated with an improved parent-infant relationship, specifically increased responsiveness and synchrony. Mother-infant dyads completing an ATVV intervention were found to have increased positive interactions during feeding, improved infant clarity of cues, and decreased levels of low responsiveness during play.

Salivary Cortisol

Feasibility for Use in Research

Title: Salivary cortisol in psychobiological research: an overview

Citation: Kirschbaum C, Hellhammer DH. Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology*. 1989;22(3):150-169. doi:10.1159/000118611

Methods: This study was a review of existing literature related to the use of salivary cortisol in research.

Results: This study had many findings due to the extensive review of previous studies, including:

- Salivary cortisol levels correlates well with levels of unbound cortisol found in the bloodstream (~90% of cortisol is bound to corticosteroid-binding globulin), and salivary cortisol levels peak approximately 1-2 minutes after a peak in blood plasma levels, indicating a minimal time lag between changes.
 - Research indicates that salivary cortisol peaks 20-30 minutes from the introduction of a stressor.
- It can be difficult to compare absolute concentrations between different labs if different assay systems are used to calculate the level of salivary cortisol, which prevents development of standardized values for salivary cortisol. (Means reported in this sample – 7-9 am: 519.03 ng/dL, 3-5 pm: 163.10 ng/dL, 8-10 pm: 71.04 ng/dL).
 - It is essential to consider the diurnal pattern of salivary cortisol when taking measurements to try to prevent skewed results.
- Many research articles indicate increased levels of salivary cortisol during pregnancy (especially during the third trimester), but there are contradictory studies that make it hard to draw firm conclusions.
- Salivary cortisol levels can be skewed in patients taking prednisolone and metyrapone due to one of the chemicals used in the assay.
- An advantage of salivary cortisol over plasma cortisol (requiring a blood draw) is that the noninvasive technique used helps prevent a potential spike in cortisol levels due to stress from the blood draw. Additionally, medically trained personnel are required to perform blood draws whereas patients can collect salivary cortisol samples themselves. Salivary cortisol is also often a cheaper method.
- Salivary cortisol has been shown to increase in response to physical stress and psychological stress (particularly situations that are deemed to be novel, unpredictable, or uncontrollable by the individual). With psychological stress, the individual's emotional processing and anticipation of noxious/detrimental events are believed to play an important role in increasing salivary cortisol levels.

Normal Ranges/Responses to Stress

Title: Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge

Citation: Kudielka BM, Hellhammer DH, Wüst S. Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. *Psychoneuroendocrinology*. 2009;34(1):2-18. doi:10.1016/j.psyneuen.2008.10.004

Methods: This study was a review of existing literature focused on identifying characteristics that may play a role in an individual's salivary cortisol levels.

Results: This study identified a number of factors that could play a role in individual differences in salivary cortisol levels, including:

- Minimal to no differences in salivary cortisol levels based on age
- Increased rise in cortisol for males compared to females when presented with a stressor
- Increased stress responses for women in the luteal phase of the menstrual cycle as compared to women in the follicular phase or those on oral contraceptives
- Increased cortisol levels with pregnancy
- Inverse correlation between birth weight and salivary cortisol responses to psychological stress
- Presence of both hypo- and hyper-reactivity of the HPA axis after exposure to chronic stress

UNC Hospitals Reference Lab Values for Cortisol

7-9 am: 100-750 ng/dL

3-5 pm: <401 ng/dL

11 pm-midnight: <100 ng/dL

no norms provided for time frames outside of these

Diurnal Pattern

Title: Do psychosocial predictors affect the following days' cortisol awakening response?

Expanding the temporal frame with which to explore morning cortisol

Citation: Proulx J, Klee D, Oken BS. Do psychosocial predictors affect the following days' cortisol awakening response? Expanding the temporal frame with which to explore morning cortisol. *Stress*. 2017;20(4):398-403. doi:10.1080/10253890.2017.1346076

Methods: 100 participants participated in this study, which was focused on mindfulness meditation. Individuals completed a variety of self-report measures (60-item NEO-FFI, salivary cortisol samples immediately upon waking/30 minutes after waking/around 4 pm/around bedtime, and the Positive and Negative Affect Schedule) on two days after their initial laboratory visit.

Results: Individuals with increased affect variability had an increased risk of being in the low-cortisol awakening response (CAR) group or having a decreased CAR slope. Additionally, individuals with increased variation in negative affect were found to have higher levels of cortisol upon waking in addition to a flatter CAR slope (smaller decline in cortisol after waking). Bedtime cortisol levels and increased waking levels of cortisol were both found to be associated with a decreased CAR slope the following day. Increased levels of neuroticism was also found to be somewhat associated with increased waking cortisol levels. This study indicates that both cortisol levels the day before and possible stressors associated with any particular given day both likely play a role in the regulation of waking salivary cortisol levels.

Title: The effects of SES on infant and maternal diurnal salivary cortisol output

Citation: Clearfield MW, Carter-Rodriguez A, Merali A-R, Shober R. The effects of SES on infant and maternal diurnal salivary cortisol output. *Infant Behav Dev*. 2014;37(3):298-304. doi:10.1016/j.infbeh.2014.04.008

Methods: This study had 32 mother-infant dyads (16 high SES and 16 low SES). Mothers were given salivary cortisol collection kits and were instructed to collect samples from themselves in their infants in the morning, afternoon, and evening.

Results: Infants were found to exhibit the same diurnal pattern of salivary cortisol levels as adults (peak highest in the morning and then a gradual decline throughout the rest of the day), with significant differences in levels found across each of the 3 time points. There was a significant difference in levels between high SES and low SES infants, with low SES infants having higher cortisol levels at all time points. Mothers also were found to exhibit the expected diurnal pattern, with significantly higher levels of salivary cortisol in the morning as compared to the afternoon or evening. Low SES mothers also had higher levels of cortisol than high SES mothers, but the difference was only statistically significant between the evening levels. There was also increased synchrony in salivary cortisol levels among high SES mother-infant dyads as compared to low SES dyads. There is some research indicating that chronically high cortisol levels may be associated with depression, which can impact mother-infant attachment and infant development. Additionally, improved attachment has been correlated with increased synchrony between cortisol levels.

Title: Long-term stability of diurnal salivary cortisol and alpha-amylase secretion patterns

Citation: Skoluda N, La Marca R, Gollwitzer M, et al. Long-term stability of diurnal salivary cortisol and alpha-amylase secretion patterns. *Physiol Behav.* 2017;175:1-8.

doi:10.1016/j.physbeh.2017.03.021

Methods: This was a secondary analysis performed on data collected for a previous study looking at the impact of stress management on both psychological and physiological indices of stress. Outcome measures were taken at baseline, 12 months after intervention, and 24 months after intervention and consisted of salivary cortisol samples, self-report questionnaires, and other medical exam items.

Results: The stress management intervention did not impact the diurnal pattern of salivary cortisol levels. There was found to a low level of long-term stability of salivary cortisol levels day to day for the same individual. This variability is thought to be a result of both the number of stressful situations a patient encounters in addition to that individuals stress reactivity. There were significant decreases in morning cortisol levels and daily cortisol levels after completing the stress management intervention, while there was an increase in the slope of cortisol levels throughout the day. This study demonstrates that even with changing absolute levels of salivary cortisol, a diurnal pattern still persists.

Overall, the presence of a diurnal pattern in salivary cortisol levels indicates the importance of taking salivary cortisol levels at a more consistent time of day when comparing samples between or within individuals over time.

Pregnancy/Postpartum

Title: Changes in the maternal hypothalamic-pituitary-adrenal axis in pregnancy and postpartum: influences on maternal and fetal outcomes

Citation: Duthie L, Reynolds RM. Changes in the maternal hypothalamic-pituitary-adrenal axis in pregnancy and postpartum: influences on maternal and fetal outcomes. *Neuroendocrinology*. 2013;98(2):106-115. doi:10.1159/000354702

Methods: This study was a review of the literature aimed at identifying changes to the HPA axis during pregnancy and postpartum.

Results: There were a variety of clinically meaningful results identified in this review, including:

- Cortisol levels rise up to threefold during the third trimester of pregnancy as the placenta releases corticotrophin-releasing hormone and estrogen stimulation of corticosteroid-binding globulin.
- Diurnal patterns of cortisol secretion are maintained during pregnancy.
- The placenta can act as a barrier in some ways but when cortisol levels become excessive (can be impacted by maternal anxiety, infection, or inflammation), the developing fetus can be exposed to increased levels of cortisol.
- Maternal plasma cortisol levels fall to normal levels during the postpartum phase, with the HPA axis being hyporesponsive for the first 3 weeks postpartum (recovery by 12 weeks postpartum typically), although cortisol levels typically remain normal due to the influence of other physiological changes.

Title: Stress questionnaires and stress biomarkers during pregnancy

Citation: Harville EW, Savitz DA, Dole N, Herring AH, Thorp JM. Stress questionnaires and stress biomarkers during pregnancy. *J Womens Health (Larchmt)*. 2009;18(9):1425-1433. doi:10.1089/jwh.2008.1102

Methods: This was an analysis performed as part of a study to assess the role of various prenatal factors on pregnancy, and mothers provided saliva and blood before 20 weeks and once again during weeks 24-29. All cortisol samples included in analyses were taken between 8 and 10 a.m.

Results: Median cortisol values were 400 ng/dL before week 20 and 550 ng/dL between weeks 24-29 of pregnancy. Life events, perceived stress, state anxiety, trait anxiety, and pregnancy-related anxiety were all found to be positively correlated with increased cortisol, while social support and active coping were inversely correlated with these variables. Reported stress was actually not strongly correlated which researchers attributed to the fact that hypocortisolism presents in some people who are dealing with chronic stress. Cortisol levels were the highest among women who were young, had not had a child yet, did not have hypertension, and had a lower BMI. The variability in absolute cortisol levels indicates that measuring the change in levels may be a more useful tool to compare responses to a treatment; additionally, other researchers have found that evening cortisol is more closely associated with other measures of stress, so that could be an important consideration with study design when collecting cortisol.

Title: Diurnal Salivary Cortisol Patterns During Pregnancy and After Delivery: Relationship to Plasma Corticotrophin-Releasing Hormone

Citation: Allolio B, Hoffman J, Linton EA, Winkelmann W, Kusche M, Schulte HM. Diurnal Salivary Cortisol Patterns During Pregnancy and After Delivery: Relationship to Plasma Corticotrophin-Releasing Hormone . *Clin Endocrinol (Oxf)*. 1990;33:279-289.

Methods: 12 pregnant women participated in this study and they provided salivary cortisol data during the third trimesters (weeks 37-40) and again in the 3-5 days following delivery. Salivary cortisol data was collected hourly from 7 a.m. to 11 p.m. with blood work being done at 7 a.m. and 7 p.m. as well. Additionally, a longitudinal study collected saliva samples to assess salivary cortisol in women across pregnancy; data was collected once every 4 weeks with samples being taken hourly from 7 a.m. to 11 p.m., starting prior to the 18th week of pregnancy and continuing until birth. Data was also collected from a group of non-pregnant volunteers as a control.

Results: The diurnal pattern of salivary cortisol was still present throughout pregnancy. However, around the 3rd trimester, total values of cortisol began to increase to almost twice the levels found in healthy controls. There were significant differences in total cortisol levels in the third trimester and in the immediate postpartum period, with levels in the postpartum period reflecting those of non-pregnant controls. These researchers concluded that the increase in cortisol levels during pregnancy is likely due to the increase in progesterone levels rather than placental corticotrophin-releasing hormone.

With all of these, studies were conducted among women with healthy or term infants. It is possible that different patterns (ex. a slower reduction in total cortisol) may be present in women who have extremely preterm infants.

Sex Differences

Title: 24-h urinary free cortisol from mid-pregnancy to 3-months postpartum: gender and parity differences and effects

Citation: Conde A, Figueiredo B. 24-h urinary free cortisol from mid-pregnancy to 3-months postpartum: gender and parity differences and effects. *Psychoneuroendocrinology*. 2014;50:264-273. doi:10.1016/j.psyneuen.2014.08.013

Methods: 26 women and 22 men who did not have any pregnancy-related, psychosocial, or medical risk participated in this study with 24 hour urinary free cortisol measures taken (patients collected all urine during the 24 hour period) during the 2nd trimester, 3rd trimester, and 3 months postpartum. These individuals also completed sociodemographic questionnaires.

Results: Salivary cortisol is more of a measure of acute responses to stress, while repeated measurements over an extended period of time (as performed in this study) can be a better way to get a sense of general HPA axis activity. Significant differences were found in the cortisol levels in the 2nd and 3rd trimester and between the 3rd trimester and 3 months postpartum (3rd trimester the highest values). Men were found to have significantly lower cortisol values during the 3rd trimester but not the other time points. Men were found to have higher cortisol levels in the postpartum period than the 2nd trimester, while women were found to have the opposite pattern. One possible explanation for the increase in cortisol in the 3rd trimester in women compared to men is the need for increased levels of cortisol to help induce labor. These researchers also hypothesized that these changes could be due to different stressors related to pregnancy in men and women, with men likely being more concerned with preparation for arrival of the infant and their partner's feelings while women are more concerned with the delivery, recovery in the postpartum period, ability to care for the child, and the baby's health. Again, it is important to consider the fact that this was performed with parents of healthy, term infants who likely do not face as much stress as NICU parents do in the postpartum period (so it is unknown if these patterns would persist with them).

Title: Sex differences in cortisol response to noxious stress

Citation: Zimmer C, Basler H-D, Vedder H, Lautenbacher S. Sex differences in cortisol response to noxious stress. *Clin J Pain*. 2003;19(4):233-239. doi:10.1097/00002508-200307000-00006

Methods: 42 men and 42 women participated in this study (all college age students). All experimental sessions took place between 5 p.m. and 8 p.m. to help eliminate discrepancies due to the diurnal pattern of cortisol. A saliva sample was collected from all participants, followed by completion of a variety of self-report measures (demographic, distress, state anxiety). Then participants provided two more salivary samples over a 60 minute rest period. Participants then completed the plunge test (submerging hand in ice water for as long as is bearable with occasional rest breaks provided at random intervals) two times, with visual analog scale ratings of the pain taken immediately afterwards, and another salivary sample taken 20 minutes after the plunge test. An additional 3 salivary samples were collected every 15 minutes following the first post test sample.

Results: There was a significant increase in salivary cortisol levels from baseline to after completion of the plunge test in both males and females, but there was a significantly larger increase in men when comparing the immediately pre and 20 minute post samples.

Researchers hypothesized that these differences could be seen as a result of differences in gonadal steroids between males and females that could impact HPA responsiveness. Researchers pointed out that it is unclear whether increased cortisol levels in males led to their ability to keep their hand submerged in ice water for longer or if the longer time with their hand submerged led to increased cortisol levels.

Title: HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: impact of age and gender

Citation: Kudielka BM, Buske-Kirschbaum A, Hellhammer DH, Kirschbaum C. HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: impact of age and gender. *Psychoneuroendocrinology*. 2004;29(1):83-98. doi:10.1016/s0306-4530(02)00146-4

Methods: Data from five previous studies were analyzed in an attempt to determine the impact of age and gender on response to various stressors. The final sample included 102 subjects (30 older adults, 41 young adults, and 31 children). All participants were exposed to the Trier Social Stress Test. Post menopausal women could not be taking any hormone replacement therapy, and premenopausal women were scheduled to come in during the luteal phase of their cycle to standardize results. Participants used a visual analog scale to rate their stress levels during the task, and salivary cortisol measurements were taken prior to initiation of the stressor and at multiple time points after exposure. Blood samples were also collected for evaluation of plasma cortisol.

Results: Completion of the stress test led to significant increases in salivary cortisol across all age/gender groups, although a gender difference emerged in the elderly group (men having higher levels than females). When looking at plasma levels of cortisol, elderly women had higher levels than elderly men (all groups had significant increases), and when looking at ACTH levels, young men had significantly higher levels than young women (all groups had significant increases). After controlling for other variables, there were no gender differences in self-reported levels of stress with the task. Researchers concluded that women have higher sensitivity to ACTH, since lower levels of ACTH hormone led to similar increases in plasma levels of cortisol. The hormonal changes that take place in women following menopause could help explain the differences seen in plasma levels of cortisol between elderly men and women (in addition to higher levels of CBG in elderly women, which would leave less unbound cortisol).

Title: Consistent sex differences in cortisol responses to psychological stress

Citation: Kirschbaum C, Wüst S, Hellhammer D. Consistent sex differences in cortisol responses to psychological stress. *Psychosom Med*. 1992;54(6):648-657. doi:10.1097/00006842-199211000-00004

Methods: Four separate studies with a total of 153 healthy subjects were analyzed with the following gender breakdowns (19 M/31 F in study 1, 23 M/14 F in study 2, 22 M/26 F in study 3, and 9M/9F in study 4). In studies 1 and 4, experimental sessions were conducted in the morning and late afternoon, while only the later afternoon time period was used in studies 2 and 3. In all 4 studies participants participated in a psychological stress test involving public speaking and mental arithmetic in front of a group of people. In study 3, participants also received an injection of corticotropin-releasing hormone and completed a bicycle ergometry

test (all on different days). In study 4 participants did not actually complete the public speaking portion; they were only told to prepare and had data collected before being told they would not have to complete it. All studies had baseline and post-intervention salivary samples taken, although the specific timing varied between studies.

Results: Males had a 3 to 3.5-fold increase in cortisol levels, while women had only a 1.5 to 2.5-fold increase in response to the public speaking and arithmetic tasks. Peak cortisol levels were reached around the same time for both sexes despite the difference in total level. With the CRH bolus and bicycle ergometry tests performed in study 3, there were no significant differences in salivary cortisol levels between males and females. In anticipation of the public speaking task, men experienced an increase in cortisol levels, while levels actually declined for women over time. These researchers concluded that some of these sex differences may be due to higher levels of CBG in women or decreased cortisol secretion in females.

There is somewhat inconclusive evidence regarding sex differences, although it does appear that there are some possible differences between male and female responses to stress. It does seem that there is a more consistent sex difference that emerges in response to psychological stress, while the relationship with physical stress is less clear. This could indicate a need to analyze data from mothers and fathers separately, particularly until there is a clearer conclusion about sex differences in cortisol responses.

Parents of Sick Children

Title: The cortisol response in parents staying with a sick child at hospital

Citation: Angelhoff C, Edéll-Gustafsson U, Mörelius E. The cortisol response in parents staying with a sick child at hospital. *Nurs Open*. 2019;6(2):620-625. doi:10.1002/nop2.245

Methods: 31 parents with children in hospital wards at 4 Swedish hospitals were included. Data was collected from parents after spending one night on the pediatric ward and 4 weeks after discharge. All parents were recruited on a Tuesday, as some research indicates cortisol awakening response values can vary on the weekend and workdays. Parents completed a demographic questionnaire and took their own saliva samples immediately after waking up the next day and again 25 min after that. Parents completed the same process 4 weeks after their child had been discharged from the hospital.

Results: The morning awakening cortisol levels were significantly lower for parents when their child was on the pediatric ward. Researchers concluded that this aligned with previous research demonstrating that lower awakening cortisol levels were present the day after a particularly stressful event. They also thought that parents may have had higher cortisol levels when at home as they now had to balance parenting of a child with a recent hospitalization with work stress.

Title: Salivary cortisol, stress, and health in primary caregivers (mothers) of children with cerebral palsy

Citation: Bella GP, Garcia MC, Spadari-Bratfisch RC. Salivary cortisol, stress, and health in primary caregivers (mothers) of children with cerebral palsy. *Psychoneuroendocrinology*. 2011;36(6):834-842. doi:10.1016/j.psyneuen.2010.11.005

Methods: 38 mothers/caregivers of children with cerebral palsy and 37 mothers/caregivers of children without any developmental problems were recruited to complete surveys related to life stress events, anthropometric data, socioeconomic status, social profile, burden of being a caregiver, perceived stress, and physical/psychological wellbeing, in addition to providing salivary cortisol samples (4 samples collected on the same day – one right after waking up, one 30 minutes after the first, one at midday before lunch, and one around 8:00 p.m.).

Results: Mothers/caregivers of children with cerebral palsy had decreased psychological/physical wellbeing. They also found that salivary cortisol levels were lower for mothers/caregivers of children with cerebral palsy, although both groups demonstrated the diurnal pattern typically seen with salivary cortisol. These researchers concluded that this was likely due to the presence of chronic stress related to caring for these children; previous research has indicated that chronic stress can lead to hyporeactivity of the HPA axis and therefore lower cortisol levels.

Both of these studies indicate that parents of sick children tend to have decreased levels of salivary cortisol, regardless of if the stress is more acute or chronic.

Neonates

Title: Longitudinal evaluation of salivary cortisol levels in full-term and preterm neonates

Citation: Bettendorf M, Albers N, Bauer J, Heinrich UE, Linderkamp O, Maser-Gluth C.

Longitudinal evaluation of salivary cortisol levels in full-term and preterm neonates. *Horm Res.* 1998;50(6):303-308. doi:10.1159/000023295

Methods: Salivary samples were taken from 40 infants every 6 hours for 3 consecutive days. 10 of the infants were healthy full-term newborns, another 10 were healthy preterm newborns, another 10 were 10 preterm newborns with lung disease or bronchopulmonary dysplasia treated with dexamethasone, and the other 10 were 10 preterm newborns with chronic lung disease or bronchopulmonary dysplasia treated with nebulized budesonide.

Results: Healthy full-term neonates had significantly higher levels of salivary cortisol than all three groups of preterm neonates. Healthy preterm neonates had significantly higher salivary cortisol levels compared to both of the other groups of preterm neonates.

Same Day Pre-Post Designs

Title: Effect of augmented reality books in salivary cortisol levels in hospitalized pediatric patients: A randomized cross-over trial

Citation: Alarcón-Yaquetto DE, Tincopa JP, Guillén-Pinto D, Bailon N, Cárcamo CP. Effect of augmented reality books in salivary cortisol levels in hospitalized pediatric patients: A randomized cross-over trial. *Int J Med Inform.* 2021;148:104404.

doi:10.1016/j.ijmedinf.2021.104404

Methods: This was a two-part study where 18 children were recruited to gauge acceptability of children's books for use in the randomized controlled trial. In the second phase, 29 children who were hospitalized received either a standard children's book or a book and tablet (augmented reality condition) for an hour. All children participated in both conditions with a 48 hour washout period in between. Cortisol levels were collected before and after each intervention, in addition to the use of a visual analogue scale to assess stress.

Results: Researchers found a significant decrease in cortisol with augmented reality books as compared to standard books; There was a negative correlation between cortisol levels and self-reported emotional stress (higher distress, higher cortisol).

Title: Salivary alpha amylase and cortisol levels as stress biomarkers in children with cerebral palsy and their association with a physical therapy program

Citation: Durán-Carabali LE, Henao-Pacheco ML, González-Clavijo AM, Dueñas Z. Salivary alpha amylase and cortisol levels as stress biomarkers in children with cerebral palsy and their association with a physical therapy program. *Res Dev Disabil.* 2021;108:103807.

doi:10.1016/j.ridd.2020.103807

Methods: 86 children with and without cerebral palsy participated in this study. For children with CP, salivary cortisol was measured before and after a physical therapy session (neurodevelopmental technique utilized) for children with cerebral palsy. Children without cerebral palsy only had salivary cortisol collected one time for a baseline comparison.

Results: For children with CP, there was a significant decrease in cortisol levels 20 minutes following PT intervention compared to basal and 5 minutes post levels.

PT session in children with CP; significant decrease in cortisol levels 20 minutes following PT

Title: The other side of the curve: examining the relationship between pre-stressor physiological responses and stress reactivity

Citation: Balodis IM, Wynne-Edwards KE, Olmstead MC. The other side of the curve: examining the relationship between pre-stressor physiological responses and stress reactivity.

Psychoneuroendocrinology. 2010;35(9):1363-1373. doi:10.1016/j.psyneuen.2010.03.011

Methods: 87 undergraduate students either participated in the Trier Social Stress Test or completed crossword puzzles. Salivary cortisol samples were collected upon arrival to the laboratory, immediately prior to beginning the intervention, immediately after the intervention, and 40 minutes following the conclusion of the intervention. Participants also completed self-report questionnaires related to psychological variables.

Results: Researchers found that after completing the Trier Social Stress Test, the experimental group had higher scores on anxiety, depression, anger, and confusion. They also had

significantly higher levels of cortisol, and males had significantly higher levels of cortisol than females. There was a 40% increase in cortisol levels pre to post intervention. However, there was no significant correlation between anxiety and cortisol levels.

Title: Stress and salivary cortisol in emergency medical dispatchers: A randomized shifts control trial

Citation: Bedini S, Braun F, Weibel L, Aussedat M, Pereira B, Dutheil F. Stress and salivary cortisol in emergency medical dispatchers: A randomized shifts control trial. *PLoS ONE*. 2017;12(5):e0177094. doi:10.1371/journal.pone.0177094

Methods: 22 phone operators at an emergency dispatch center provided salivary cortisol samples on a non-work day and on three different types of work days (incoming emergency calls, dispatch, re-assessment). Samples were taken every 15 minutes for 2 hours for each shift and at the same time of day for each shift. Perceived stress was also assessed.

Results: In emergency responders, there were significantly higher cortisol levels during incoming call shift as compared to dispatch or reassessment. It was also significantly greater for men at this time point, and cortisol was associated with perceived stress.

Title: Randomized controlled evaluation of the effects of cognitive-behavioral stress management on cortisol responses to acute stress in healthy subjects

Citation: Gaab J, Blättler N, Menzi T, Pabst B, Stoyer S, Ehler U. Randomized controlled evaluation of the effects of cognitive-behavioral stress management on cortisol responses to acute stress in healthy subjects. *Psychoneuroendocrinology*. 2003;28(6):767-779. doi:10.1016/s0306-4530(02)00069-0

Methods: 48 participants underwent the Trier Social Stress Test (TSST) either before or after the completion of a stress management course. Saliva samples were taken before and after completion of the TSST and at 10, 20, 30, 45, and 60 minutes after completion. Participants also completed a number of self-report measures related to stress.

Results: These researchers investigated the use of a cognitive behavioral stress management intervention before or after the TSST in healthy male college students. They found a significant increase in salivary cortisol for both groups, but it was attenuated in cognitive behavioral group.

Title: Cortisol response to the Trier Social Stress Test in pregnant women at risk for postpartum depression

Citation: Deligiannidis KM, Kroll-Desrosiers AR, Svenson A, et al. Cortisol response to the Trier Social Stress Test in pregnant women at risk for postpartum depression. *Arch Womens Ment Health*. 2016;19(5):789-797. doi:10.1007/s00737-016-0615-7

Methods: 44 pregnant women participated in this study (24 at risk for postpartum depression, 20 not at risk). These women completed the TSST and provided multiple salivary cortisol samples in addition to completing self-report measurements of depression and anxiety.

Results: In this study, researchers completed the TSST in women at risk for postpartum depression and with healthy controls. There was no correlation between EPDS and baseline cortisol; there was also no difference in cortisol values over time for these groups.

Title: Exercise-induced responses in salivary testosterone, cortisol, and their ratios in men: a meta-analysis

Citation: Hayes LD, Grace FM, Baker JS, Sculthorpe N. Exercise-induced responses in salivary testosterone, cortisol, and their ratios in men: a meta-analysis. *Sports Med.* 2015;45(5):713-726. doi:10.1007/s40279-015-0306-y

Methods: This study was a synthesis and analysis of 21 articles related to salivary cortisol and testosterone levels around the time of exercise in men.

Results: This study investigated exercise responses in men and found that aerobic and resistance training led to significant increases in salivary cortisol.

Title: Distraction coping predicts better cortisol recovery after acute psychosocial stress

Citation: Janson J, Rohleder N. Distraction coping predicts better cortisol recovery after acute psychosocial stress. *Biol Psychol.* 2017;128:117-124. doi:10.1016/j.biopsycho.2017.07.014

Methods: 59 adults completed this study, which consisted of resting for 30 minutes, completing the TSST, and then resting for an additional 60 minutes. Saliva samples were taken immediately before beginning the TSST, immediately after completion of the TSST, and 10/20/30/45/60 minutes after completion of the TSST. Participants also completed measures related to state coping, trait coping, chronic stress, depressive symptoms, and rumination.

Results: This study found that the TSST led to a significant increase in salivary cortisol (M>F) and that mean cortisol peaked 10 minutes after completion of the TSST.

Title: A randomized, controlled, single-blinded study on the impact of a single rhythmical massage (anthroposophic medicine) on well-being and salivary cortisol in healthy adults

Citation: Kanitz JL, Reif M, Rihs C, Krause I, Seifert G. A randomised, controlled, single-blinded study on the impact of a single rhythmical massage (anthroposophic medicine) on well-being and salivary cortisol in healthy adults. *Complement Ther Med.* 2015;23(5):685-692. doi:10.1016/j.ctim.2015.07.008

Methods: 101 participants completed this study, where they completed the TSST and then received 30 minutes of rhythmical massage (or sham massage) and 20 minutes of rest. Salivary cortisol samples were collected before the TSST, after the TSST, after 20 minutes of massage, after 10 more minutes of massage, and after 30 minutes of rest. Participants also completed a mental state scale, a list of somatic complaints, and visual analogue scales related to various mood states.

Results: This study used one rhythmical massage session in adults. These adults had normal stress scores at baseline, and there were no statistically different changes in cortisol between groups.

Title: Sex differences in salivary cortisol reactivity to the Trier Social Stress Test (TSST): A meta-analysis

Citation: Liu JJW, Ein N, Peck K, Huang V, Pruessner JC, Vickers K. Sex differences in salivary cortisol reactivity to the Trier Social Stress Test (TSST): A meta-analysis.

Psychoneuroendocrinology. 2017;82:26-37. doi:10.1016/j.psyneuen.2017.04.007

Methods:

Results: This study investigated sex differences in responses to the TSST. They found that cortisol levels peaked 13 minutes after the completion of the TSST (based on meta-analysis studies). There was no significant effect between genders on salivary cortisol at baseline, but there was a significant difference at peak (M>F). Women not on oral contraceptives had higher levels of cortisol than women on them.

Liu et al – TSST sex differences; peak cortisol post TSST 13 min (meta-analysis); no significant effect on salivary cortisol at baseline, significant difference at peak with men > women; women not on oral contraceptive higher levels than women on oral contraceptives.

Title: Differences in salivary alpha-amylase and cortisol responsiveness following exposure to electrical stimulation versus the Trier Social Stress Tests

Citation: Maruyama Y, Kawano A, Okamoto S, et al. Differences in salivary alpha-amylase and cortisol responsiveness following exposure to electrical stimulation versus the Trier Social Stress Tests. *PLoS ONE*. 2012;7(7):e39375. doi:10.1371/journal.pone.0039375

Methods: 149 adults participated in this study, and all participants were exposed to both the TSST and electrical stimulation (on separate days). Saliva samples were taken to measure cortisol and alpha-amylase immediately before, immediately after, and 20 minutes after the intervention. Participants also completed self-report measures related to mental state and had heart rate assessed throughout the interventions.

Results: There was a significantly greater increase in reaction to the TSST than e stim, in addition to a prolonged responsiveness after TSST (sig. diff. baseline and immediately after, immediately after and 20 min after). There was a significant relationship between tension-anxiety and salivary cortisol after e stim but not TSST. There were also no sex differences.

Title: Trained men show lower cortisol, heart rate, and psychological responses to psychosocial stress compared with untrained men

Citation: Rimmelle U, Zellweger BC, Marti B, et al. Trained men show lower cortisol, heart rate and psychological responses to psychosocial stress compared with untrained men.

Psychoneuroendocrinology. 2007;32(6):627-635. doi:10.1016/j.psyneuen.2007.04.005

Methods: 44 men (22 elite athletes, 22 untrained men) completed the TSST, followed by 90 minutes of rest in the laboratory. Salivary cortisol samples were taken before and 10/20/30/45/60/90 minutes after the intervention. Heart rate measurements were assessed every 5 seconds throughout the duration of the TSST and for 2 minutes after its completion. Subjects also completed self-report measures related to psychological state and personality traits.

Results: After completing the TSST in trained men and untrained men, there were significant increases in salivary cortisol in both groups but trained men had significantly lower responses. There were worse mood levels in untrained group and a significant increase in state anxiety in both groups.

Title: The perioperative dialogue reduces postoperative stress in children undergoing day surgery as confirmed by salivary cortisol

Citation: Wennström B, Törnåge C-J, Nasic S, Hedelin H, Bergh I. The perioperative dialogue reduces postoperative stress in children undergoing day surgery as confirmed by salivary cortisol. *Paediatr Anaesth.* 2011;21(10):1058-1065. doi:10.1111/j.1460-9592.2011.03656.x

Methods: 93 children undergoing surgery participated in this study and either received standard preoperative care, standard preoperative care in addition to preoperative information, or standard care in addition to a perioperative dialogue with an anesthesiology nurse. Cortisol was measured on the morning of surgery, before the induction of anesthesia, and post-operatively.

Results: This study investigated the impact of utilizing a perioperative dialogue with children the day of surgery. They found significantly lower levels of salivary cortisol post-op in PD group; decreased from baseline to post-op in 96% in PD group but only 63-72% in other groups

Title: Implicit motives and children's salivary cortisol reactivity to an adapted version of the Trier Social Stress Test for Children (TSST-C)

Citation: Spengler B, Hofer J, Busch H, Dzionsko I, Emslander V. Implicit motives and children's salivary cortisol reactivity to an adapted version of the Trier Social Stress Test for Children (TSST-C). *Pers Individ Dif.* 2020;162:110010. doi:10.1016/j.paid.2020.110010

Methods: 89 children completed the an adapted version of the TSST, Salivary cortisol samples were taken 10 minutes after arriving in the laboratory, after completion of a picture story exercise, after completion of the TSST, after completion of questionnaires, and after 10 and 20 minutes of playing quiet games. Self-reported affect was assessed immediately before and after completion of the TSST.

Results: After completing the TSST in children, there was a significant decrease in self-reported affect. They also found that cortisol peaked 20 min after onset of social-evaluative stressor and that it 20 additional minutes to return to baseline.

Salivary cortisol has been assessed in many studies using pre-post same-day designs following exposure to a stress-inducing or stress-relaxing intervention, so it is both feasible and acceptable to use this method in future studies. Samples are typically taken immediately before and after the intervention, in addition to following a rest period. Significant changes in salivary cortisol levels have been seen using this method, with peak salivary cortisol levels tending to occur 10-20 minutes after completion of exposure to a stress-inducing situation.

Relationship with Perceived Stress

Title: Effects of a mindfulness-based intervention on mindfulness, stress, salivary alpha-amylase, and cortisol in everyday life

Citation: Aguilar-Raab C, Stoffel M, Hernández C, et al. Effects of a mindfulness-based intervention on mindfulness, stress, salivary alpha-amylase and cortisol in everyday life. *Psychophysiology*. 2021;58(12):e13937. doi:10.1111/psyp.13937

Methods: 74 medical students participated in this study shortly before taking their first exams. Participants either attended a stress management/mindfulness course or sham classes. Cortisol and alpha-amylase were measured through saliva samples over two days pre- and two days post-intervention (6 sampling times throughout the day).

Results: Researchers found no correlation between cortisol and perceived stress; they did find that mindfulness was associated with decreased perceived stress but not cortisol.

Title: Effects of Hatha yoga and African dance on perceived stress, affect, and salivary cortisol

Citation: West J, Otte C, Geher K, Johnson J, Mohr DC. Effects of Hatha yoga and African dance on perceived stress, affect, and salivary cortisol. *Ann Behav Med*. 2004;28(2):114-118. doi:10.1207/s15324796abm2802_6

Methods: 69 undergraduate students enrolled in either Hatha yoga, African dance, or biology participated in this study. Participants provided 4 salivary cortisol samples before class and 4 samples after class. They also completed self-report measures related to perceived stress and affect.

Results: This investigated the use of Hatha yoga and African dance to reduce stress. Both reduced perceived stress and negative affect, with an increase in positive affect in dance group; cortisol significantly increased in dance and significantly decreased in yoga immediately post collection.

Current research findings are mixed regarding the relationship between perceived stress and salivary cortisol levels, with the two studies above finding no relationship, an inverse relationship, and a direct relationship between the two variables.

Significant Changes

Title: Classification criteria for distinguishing cortisol responders from nonresponders to psychosocial stress: evaluation of salivary cortisol pulse detection in panel designs

Citation: Miller R, Plessow F, Kirschbaum C, Stalder T. Classification criteria for distinguishing cortisol responders from nonresponders to psychosocial stress: evaluation of salivary cortisol pulse detection in panel designs. *Psychosom Med.* 2013;75(9):832-840.

doi:10.1097/PSY.0000000000000002

Methods: 504 participants either completed the TSST or a placebo version of the TSST. Saliva samples were taken 6 minutes before, 16 minutes after, 25 minutes after, 35 minutes after, 45 minutes after, and 55 minutes after completion of the TSST.

Results: In a previous study responders were determined to have a change of 2.5 nmol/L, but this study found responders to be a change of 1.5 nmol/L based on the TSST. They found that this classification reduces false negative classifications to 9.7% with virtually no false positives. Additionally, it was determined that a percent change of 15.1% can be used to distinguish responders from non-responders.