Effects of tactile-kinesthetic stimulation on feeding and weight of moderate and late preterm neonates

CURRENT STATUS: UNDER REVIEW

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DOI:
10.21203/rs.3.rs-16398/v1

SUBJECT AREAS
Pediatrics

KEYWORDS
Preterm neonates, tactile-kinesthetic stimulation, feeding intolerance, and weight.
Abstract
Background: Owing to immaturity of their body systems, preterm neonates are susceptible to feeding intolerance, slowed growth and long hospitalization periods among others. Tactile-kinesthetic stimulation, a moderate pressure of the skin and joint movement has been widely researched on among preterm neonates; the studies have predominately focused on weight gain and the potential underlying mechanism. This study focuses on effect of tactile-kinesthetic stimulation on amount of feeds, feeding intolerance and weight of moderate and late preterm neonates. Methods: A quasi-experimental study comprising 72 preterm neonates born at 28 to <37 weeks gestation age (GA) was conducted. Subjects were divided into two groups; control and tactile-kinesthetic stimulation (TKS). Neonates in the TKS group received massage for 15 minutes per session; 3 times a day for 10 days while control group had standard nursery care. Neonates’ amount of feeds and signs of feeding intolerance were assessed on days 3, 10, 17, and 23 of life while weight was measured on alternate days stating on day 3 up to day 23. Mann Whitney U test, Student t-test, Chi-square test, and Fisher’s exact test were used to determine whether there was any difference in feeding parameters and weight between TKS and control groups. Results: Although amount of feeds didn’t differ between the groups, TKS group neonates had fewer episodes of feeding intolerance compared to those in control group on the 10th (p = .03), 17th (p = .00), and 23th (p = .00) day of life. Moderate preterm neonates didn’t differ in weight gain however; late preterm neonates in TKS group had significantly more weight gain than the control. Conclusion: Tactile-kinesthetic stimulation reduces feeding intolerance and enhance weight gain in moderate preterm neonates. Key words: Preterm neonates, tactile-kinesthetic stimulation, feeding intolerance, and weight. Trial registration: ClinicalTrials.gov NCT04287322, registered on 27/02/2020. Retrospectively registered.

Background
A steady increase in the rate of preterm births has been witnessed in the last decade constituting 11.1% of 135 million live births worldwide, the majority of which occur in sub-Saharan Africa. In Kenya, the prevalence of preterm birth stands at 6.7% as a result of demographic change, infertility treatment, increased maternal age, multiple births, and increased rate of obesity (1-3).
Immature digestive system of preterm neonates induces feeding intolerance resulting in increased risk of infection, inability to maintain enteral feeds, and weight loss\(^4, 5\); in addition to necrotizing enterocolitis - a leading cause of morbidity and mortality of preterm neonates\(^6\). With priority to promptly achieve full enteral feeds for the preterm neonates, signs of feeding intolerance are a great set back in care of the preterm neonates. Although in high-income countries supplemental parenteral nutrition is commonly given to preterm neonates with difficulty in tolerating adequate enteral feeds\(^7\) it is way too costly for a majority of the population in Kenya and therefore not used in Moi Teaching & Referral Hospital (MTRH). The preterm neonates are rather put on maintenance fluid (Ringer’s lactate/5% dextrose) despite its lack of optimal nutritional requirements for the neonates. Inadequate nutrition coupled with feeding intolerance increases length of time to reach full enteral feeding; and the latter has been associated with poorer mental outcome in preterm neonates at 24 months corrected age\(^4\). Through parasympathetic activity massage accelerates peristalsis, decrease abdominal distension, accelerate bowl transit time, increase frequency of stooling, and decrease frequency of vomiting\(^4, 5\).

Optimal physical growth is important for normal growth and development of preterm neonates. Rapid growth during hospitalization allows early discharge, rapid adaptation to home environment, and emotional attachment to the mother. Poor growth indices have been associated with lower neurodevelopmental and growth outcomes later in life\(^4\). Enhancing retention and digestion of feeds for growth and development is a core component in care of preterm neonates. Weight being a critical criterion for discharge from neonatal unit is a major problem. In many instances, insufficient weight gain is the main reason for prolonged hospitalization, high cost care, and related problems\(^8\). At MTRH Special Care Nursery (SCN) the hospital policy allows neonates to be discharged after attaining 1700g. Strategies that enhance weight gain not only reduce the cost of care but allow mother to bond with her infant in a relaxed home environment. Tactile-kinesthetic stimulation (TKS), a form of massage therapy involves moderate-pressure stroking of the neonate and movements of large joints. Through parasympathetic activity massage and kinesthetic movements (extension and flexion of
joints) increases secretion of digestive hormones (insulin, gastrin and gastric fluid), increases mean enteral intake and weight gain \(^{(9, 10)}\). Massage therapy is safe and tolerated by preterm neonates; the neonates are more responsive to tactile stimulation than other sensory modalities due to early maturation of the tactile system and the large surface area of the skin \(^{(11)}\).

Although studies have demonstrated more enteral intake, and greater weight gain in preterm neonates as a result of TKS \(^{(1, 12, 13)}\) little has been done on feeding intolerance and comparison of weight between moderate and late preterm neonates. The purpose of this study was to determine effects of TKS on feeding and weight of moderate and late preterm neonates.

**Methods**

**Study design**

A quasi-experimental design was carried out to assess effects of tactile-kinesthetic stimulation on feeding parameters and physical growth of preterm neonates. The study involved two groups; control and tactile-kinesthetic stimulation (intervention) groups.

**Study setting**

The study was conducted at a level II Special Care Nursery (American Academy of Pediatrics, 2012) \(^{(14)}\) of Moi Teaching & Referral Hospital (MTRH), an academic hospital in the Western region of Kenya. It has 70-bed capacity, 11 incubators, and 54 cots. It provides care to preterm and moderately ill neonates who do not require mechanical ventilation.

**Study population**

The study population comprised preterm neonates admitted at SCN of MTRH. The SCN has an average of 90 neonates of whom majority (75%) are preterm (hospital records, 2017).

**Sample size**

Using Epi-Info software and main output variable as mean weight gain which was compared in the two groups, sample size was estimated to be 36 participants in each group, totaling to 72 participants for the study to detect average weight gain of 27±3 grams per day among the interventional group compared to average weight gain of 25±3 grams per day among the control group \(^{(12)}\). Thus, change
of average weight gain by two grams was detected. The estimated sample size was made at assumption of 95% confidence level and 80% power of study.

**Eligibility criteria;** neonates who fulfilled the following criteria comprised the study participants; a) on breast milk or formula feeds, b) born 28 to 37 weeks gestational age, c) ≥1000grams. The gestation was limited to ≥ 28 weeks and ≥1000grams based on significant neonatal mortality rate in neonates born before 28 weeks gestation and/or weighing <1000grams in MTRH (15). Neonates were excluded from the study if they; a) were critically ill and those on continuous positive airway pressure (CPAP) or b) had neonatal infections including severe sepsis or necrotizing enterocolitis.

**Sampling method**

Consecutive sampling was used to recruit preterm neonates for the study on day 3 of the neonate’s life. Study participants that met the eligibility criteria were consecutively selected in order of appearance according to their convenient accessibility. The first study participant was recruited in the control group while the subsequent with similar characteristics in the intervention group, this was continued until the desired sample size was reached.

**Study tool**

A study tool developed by the authors after thorough review of related literature on effects of TKS (1, 16) was used to collect data on a) neonates characteristics including gender, gestational age, and clinical risk index for babies (CRIB II) score; b) amount of feeds, signs of feeding intolerance (gastric residual >50% of previous feed as per MTRH hospital practices, abdominal distension and vomiting), and weight in grams. Data on feeding characteristics were analyzed on day 3, 10, 17, and 23 while that for weight on alternate days starting from day 3 up to day 23.

**Study tool validity & reliability**

Tools were reviewed for content validity by 5 experts in the field of pediatric nursing and suggestions made were incorporated into the tools. Medical records were used to gather information on amount of feeds and signs of feeding intolerance. Inter-rater reliability using two observers (research assistant and staff nurse at SCN) were done for weight recording. Notably, there was no variation in weight
recording between the two observers. A pilot study was carried out on 12 preterm neonates (four in each group) at MTRH hospital prior to data collection to test the feasibility of the study. Neonates included in the pilot study were excluded from the main study. There were no amendments made to the study tool.

**Study procedures**

A researcher was trained on TKS by a specialized pediatric nurse to ensure moderate pressure is applied on the body surface in a manner to cause effect and not harm the neonate. The training was conducted in three sessions each lasting two hours. The first and second session were conducted in the skills laboratory while the third session at MTRH hospital. The first session comprised review of massage procedure and watching the video. The second session was return demonstration on a dummy. The third session was practice on preterm neonates to achieve competency. Two research assistants (RA’s - Bachelor of Science in nursing intern) were trained on data entry. The RA’s were blinded to aim of study, group assignment, and interventions received by the preterm neonates. In addition RA’s had no access to study data; completed assessment tools, and notes.

**Control group**

Neonates in this group didn’t receive any specific stimulation rather had standard care of the SCN.

**Tactile-Kinesthetic Stimulation (TKS) group**

The group received TKS in addition to standard care of SCN.

Tactile-kinesthetic stimulation involved three sessions per day; morning, afternoon, and evening for 10 days starting day 3 of life (initial contact). After thorough hand scrubbing, the researcher placed her warmed hands on the preterm neonate’s body. Access ports were used for neonates in the incubator to prevent hypothermia. The preterm neonates were on a cardiorespiratory monitor for physiological monitoring with set alarm to detect deviation from normal that would warrant discontinuation of the TKS. The stimulation was given 1-2 hours after feeding. A small amount of sunflower oil was used for TKS to decrease injurious friction between surfaces (providers’ palms and neonate’s skin) and was removed with cotton after the stimulation. Stimulation was temporarily stopped if the neonate started crying or passed urine or stool. The stimulation was continued when
the neonate regained stability. The 15-minutes stimulation included three standardized 5 minutes phases.

The phases were as follows:

**Phase 1:** Preterm neonates were placed in prone position. Moderate pressure (sufficient to produce slight skin indentation or slight skin color change from pink to white) was used to provide 12 strokes with palms of the hands, each stroke lasting 5 seconds. The strokes were provided in each area as follows: (a) head - from forehead hairline over scalp down to neck with alternate hands; (b) neck - from midline outwards with both hands simultaneously; (c) shoulders - from midline outwards with both hands simultaneously, and (d) back - from nape of neck down to buttocks, long stroke with alternate hands.

**Phase 2:** The preterm neonates were placed in supine position. Twelve moderate pressure strokes with palms of the hands, 5 seconds each, were provided in each area as follows: (a) forehead - from midline, outwards with both hands simultaneously; (b) cheeks - from side of nose, with both hands simultaneously in rotating and clockwise direction; (c) chest - ‘butterfly’ stroking from midline upwards, outwards, downwards and inwards back to initiating point; (d) abdomen - from the appendix, in a clockwise direction around abdomen avoiding the epigastrium and probes, with gentle strokes; (e) upper limbs (each separately) - from shoulders to wrist using alternate hands for stroking; (f) lower limbs (each separately) - from hips to ankles using alternate hands for stroking; (g) palms - from wrist to finger tips using alternate hands for stroking; and (h) soles - from heel to toe tips using alternate hands for stroking.

**Phase 3:** Kinesthetic stimulation was done for 5 minutes. The intervention comprised five passive flexion and extension movements of each large joint (shoulder, elbow, wrist, hip, knee, and ankle) for two seconds.

The massage therapy protocol was adopted from Mathai 2001 (17) a modification of Field et al. (1986) protocol for medically stable preterm neonates.

**Data management and analysis**

Data from study tools was coded and entered into Statistical Package for the Social Sciences (SPSS)
version 20 database that was created for the study. Study tools were visually checked for completeness and accuracy before data entry. Descriptive statistics were computed for the baseline clinical data of the study participants. Chi-square test and student t-test were used to show relationship between variables (gender, GA, Apgar score, birth weight, and CRIB II score) between TKS and control groups. Means and (% confidence intervals, standard deviations (SDs) and interquartile ranges (IQR) were computed to describe dependent variables; amount of feed, signs of feeding intolerance, duration to attaining full breastfeeding and weight. Mann Whitney U test, Student t-test, Chi-square test, and Fisher’s exact test were used to determine whether there was any difference between TKS and control groups in amount of feeds and signs of feeding intolerance. Student t-test and Mann Whitney U test were used to determine whether a difference existed in weight of moderate and late preterm neonates between the two groups.

Results
Seventy two participants (36 in each group) were recruited to the study on day 3 of life. Twelve participants; six in each group did not complete the study; six participants in TKS group and four in control group were discharged during study period while two participants in control group died before completion of study. The sixty participants all singletons (30 each in group) were followed up to completion of the study (21 days). Chi-square test and student t-test were used to analyze baseline clinical characteristics; results showed no statistical difference between TKS and control groups as regards gender, Apgar score, birth weight, length, head circumference and temperature at birth. A statistically significant lower mean gestational age ($p = .03$) and higher mean CRIB II score ($p = .02$) were observed in TKS group compared to control group. Table 1 summarizes the baseline clinical characteristics of the participants.

Mann Whitney U test and Student t-test were used to analyze amount of feeds between TKS and control groups and the results showed the amount of feeds didn’t differ between the two groups. Although TKS group attained full breastfeeding (no gavage or cup feeding) a day earlier than the control group, the difference was not statistically significant. Chi-square test and Student t-test were used to analyze feeding intolerance (gastric residual, abdominal distension, and vomiting) and the
findings showed no difference at initial contact; occurring among half of the participants in TKS group and 63% in the control group. However, on the 10th (p = .03), 17th (p = .00), and 23rd (p = .00) day of life TKS group had significantly fewer episodes of feeding intolerance than control group. Table 2 and 3 summarizes the feeding characteristics of the participants.

Analysis of mean weight for moderate preterm neonates using Student t-test and Mann Whitney U test showed at initial contact (3rd day of life) TKS had 1164.0±160.3 while that for control group was 1262.3±131.7 and at the end of the study (23rd day of life) was 1555.4±231.0 and 1581.9±195.2 respectively. Weight didn’t differ between the two groups. However, a statistically significant difference was observed in late preterm neonates between the two groups on 15th (p = .05), 21st (p = .04) and 23rd (p = .02) day of life where TKS group neonates had more weight than control (Table 3a).

Discussion

In the recent years the focus on preterm care has shifted from increasing survival to minimizing morbidity and improving neurodevelopmental outcomes (18). Sensory stimulation such as tactile-kinesthetic stimulation (TKS) provides a platform for learning during infancy with potential to improve the outcomes of preterm neonates (19-21).

Similar to most studies (22-26), we found no difference in mean enteral intake between the intervention and control groups. Few studies (27, 28) have reported more enteral intake with TKS compared to control. For instance, Fontana et al. (2018) demonstrated a higher consumption of breast milk at discharge among preterm neonates receiving massage therapy compared to control (27).

In additional to monitoring amount of feeds intake, signs of feeding intolerance are key in nutritional management of preterm neonates. Although the definition of feeding intolerance varies with population and feeding practices, the term has been based historically on presence of increased amount of gastric aspirate, abdominal distention, and vomiting (16, 29, 30). Preterm neonates in the interventional group had significantly fewer episodes of signs of feeding intolerance compared to the
control group. Despite several studies assessing effects of tactile-kinesthetic stimulation on preterm neonates, we found none has explored feeding intolerance. However, studies\(^{(5, 16, 31)}\) on abdominal massage of preterm neonates have reported similar findings demonstrating increased peristalsis (gastric motility) from first day of massage therapy as the underlying mechanism\(^{(16)}\).

Moderate preterm neonate’s weight didn’t differ between interventional and control groups in this study, however, this could be attributed to subnormal growth that occurs in such neonates\(^{(31)}\).

Notably, similar to most studies\(^{(1, 8, 11, 12, 17, 22, 23, 32-36)}\), we found greater weight gain in late preterm neonates in the interventional group compared to the control group. The underlying mechanism for weight gain has been suggested to be increased vagal activity leading to heightened gastric motility, and increased secretion of digestive enzymes - insulin, insulin growth factor – 1, gastrin, and ornithine decarboxylase enzyme\(^{(9, 37-39)}\).

Early establishment of breastfeeding helps preterm neonates bonding, warmth, cuddling, and ultimately growth and skill development. Intervventional group preterm neonates attained full breastfeeding a day earlier than those in control group although the difference was not statistically significant.

**Conclusion**

Preterm neonates that receive tactile-kinesthetic stimulation have fewer signs of feeding intolerance.

In addition, tactile-kinesthetic stimulation enhances weight gain in late preterm neonates although the same is not observed in moderate preterm neonates. Our findings indicate the need for teaching mothers of stable preterm neonates on tactile-kinesthetic stimulation and its incorporation as a care strategy of preterm neonates in

**Abbreviations**

CPAP – Continuous positive airway pressure
CRIB II – Clinical risk index for babies
GA – Gestation age
IQR – Interquartile ranges
IREC – Institutional Research & Ethics Committee
Declarations

**Ethics approval and consent to participate statement:** the study was approved by the Institutional Research & Ethics Committee (IREC) of University’s College of Health Sciences and Moi Teaching & Referral Hospital – Kenya. Following informed written consent from their mothers, neonates were recruited to the study. In addition the study is registered under ClinicalTrials.gov trial registration number NCT04287322.

**Consent for publication:** not applicable.

**Competing interests:** authors have nothing to disclose with respect to any affiliation or financial agreement that would lead to a conflict of interest.

**Authors’ contributions:** EMN, NSB, GEM, and FOE participated in developing the study protocol, monitoring data collection process and analyzing the data, writing the manuscript. All authors read and approved the final manuscript.

**Funding:** no funding was received.

**Acknowledgements:** we thank the health care professionals at nursery unit of MTRH– Kenya and research assistants for support during the study. We are also very grateful to mothers and their preterm neonates for their participation in the study.

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Tables

| Baseline Characteristics | TKS Group [n=30] | Control Group | Significance |
|--------------------------|-------------------|---------------|--------------|

15
| 1.1 Gender | [n=30] |  |  |  |
|-------------|--------|---|---|---|
| Male        | 16     | 53.3 | 11 | 36.7 |
| Female      | 14     | 46.7 | 19 | 63.3 |

\[ X^2 = 1.684 \quad p = 0.194 \]

| 1.2 Gestational age (weeks) |  |  |  |  |
|-----------------------------|--------|---|---|---|
| Min-Max                     | 28-35  | 28-35 |  |  |
| Mean±SD                     | 30.3±2.6 | 31.8±2.4 |  |  |

\[ t = 2.198 \quad p = 0.032* \]

| 1.3 Apgar score 1 minute |  |  |  |  |
|--------------------------|--------|---|---|---|
| 0-3                      | 3      | 10.0 | 3  | 10.0 |
| 4-6                      | 11     | 36.7 | 9  | 30.0 |
| 7-10                     | 16     | 53.3 | 18 | 60.0 |
| Min-Max                  | 1-9    | 3-10 |  |  |
| Mean±SD                  | 6.3±2.1 | 6.8±1.9 |  |  |

\[ t = 0.950 \quad p = 0.346 \]

| 1.4 Apgar score 5 minutes |  |  |  |  |
|---------------------------|--------|---|---|---|
| 0-3                       | 1      | 3.3 | 0  | 0.0 |
| 4-6                       | 9      | 30.0 | 6  | 20.0 |
| 7-10                      | 20     | 66.7 | 24 | 80.0 |
| Min-Max                   | 2-10   | 4-10 |  |  |
| Mean±SD                   | 7.2±1.8 | 7.9±1.6 |  |  |

\[ t = 1.498 \quad p = 0.140 \]

| 1.5 Birth weight (grams)  |  |  |  |  |
|---------------------------|--------|---|---|---|
| Min-Max                   | 1000.0-1500.0 | 1000.0-1500.0 |  |  |
| Mean±SD                   | 1273.7±167.8 | 1304.2±168.8 |  |  |

\[ t = 0.703 \quad p = 0.485 \]

| 1.6 Length (cm)           |  |  |  |  |
|----------------------------|--------|---|---|---|
| Min-Max                   | 34.5-44.0 | 32.0-44.5 |  |  |
| Mean±SD                   | 39.3±2.5 | 39.8±3.0 |  |  |

\[ t = 0.704 \quad p = 0.484 \]

| 1.7 Head circumference (cm)|  |  |  |  |
|-----------------------------|--------|---|---|---|
| Min-Max                     | 25.0-30.0 | 26.0-32.0 |  |  |
| Mean±SD                     | 28.1±1.2 | 28.4±1.4 |  |  |

\[ t = 0.816 \quad p = 0.418 \]

| 1.8 Temperature at birth (°C) |  |  |  |  |
|-------------------------------|--------|---|---|---|
| Min-Max                       | 33.4-37.8 | 33.4-37.0 |  |  |
| Mean±SD                       | 35.8±1.1 | 35.7±0.8 |  |  |

\[ t = 0.392 \quad p = 0.696 \]

| 1.9 Clinical risk index for babies (CRIB II) |  |  |  |  |
|----------------------------------------------|--------|---|---|---|
| 7-8                                          | 9      | 30.0 | 14 | 46.7 |
| 9-10                                         | 4      | 13.3 | 7  | 23.3 |
| 11-12                                        | 8      | 26.7 | 5  | 16.7 |
| 13-14                                        | 7      | 23.3 | 4  | 13.3 |
| 15-16                                        | 2      | 6.7  | 0  | 0.0  |
| Min-Max                                      | 7-16   | 7-14 |  |  |
| Mean±SD                                      | 10.9±2.7 | 9.5±2.1 |  |  |

\[ t = 2.311 \quad p = 0.024* \]
### Table 2: Amount of feeds

| Amount of feed (mls) | TKS Group [n=30] | Control Group [n=30] | Significance |
|---------------------|------------------|----------------------|--------------|
| Day 3               |                  |                      |              |
| Min-Max             | 10.0-191.0       | 0.0-201.0            | Z=1.40       |
| Median (Q1-Q3)      | 71.5(33.8-110.3) | 52.0(11.8-87.0)     | p = .16      |
| Day 10              |                  |                      |              |
| Min-Max             | 114-332          | 0-344                | Z=0.39       |
| Median (Q1-Q3)      | 238.0(188.5-264.0)| 220.0(170.3-280.0)  | p = .69      |
| Day 17              |                  |                      |              |
| Min-Max             | 092-348          | 30-360               | Z=1.28       |
| Median (Q1-Q3)      | 288.0(265.5-320.0)| 256.0(212.8-323.5)  | p = .20      |
| Day 23              |                  |                      |              |
| Min-Max             | 192-368          | 0-400                | Z=0.87       |
| Median (Q1-Q3)      | 332.0(288.0-360.0)| 312.0(266.3-360.0)  | p = .38      |

2.2 Duration to attaining full breastfeeding (days)

| Min-Max | 24-68 | 0-51 | t=0.241 |
| Mean±SD  | 30.4±10.7 | 29.1±9.6 | P = .810 |

Z: Mann Whitney test t: Student t-test *significant at p≤0.05

### Table: 3 Signs of feeding intolerance

| Signs of feeding intolerance (gastric residual, abdominal distension, vomiting) | TKS [n=30] | Control [n=30] | Significance |
|-----------------------------------------------------------------------------|------------|----------------|--------------|
|                                                                             | No. | %  | No. | %  |               |
| Day 3                                                                        |     |   |     |   |               |
| Absent                                                                       | 15  | 50.0 | 11  | 36.7 | X^2=1.09(   |
| Present                                                                      | 15  | 50.0 | 19  | 63.3 | P=0.297       |
| Day 10                                                                       |     |   |     |   |               |
| Absent                                                                       | 23  | 76.7 | 15  | 50.0 | X^2=4.59(   |
| Present                                                                      | 7   | 23.3 | 15  | 50.0 | P=0.032*     |
| Day 17                                                                       |     |   |     |   |               |
| Absent                                                                       | 30  | 100.0 | 21  | 70.0 | FE_P=0.0(    |
| Present                                                                      | 0   | 0.0  | 9   | 30.0 |               |
| Day 23                                                                       |     |   |     |   |               |
| Absent                                                                       | 29  | 96.7 | 21  | 70.0 | X^2=7.68(   |
| Present                                                                      | 1   | 3.3  | 9   | 30.0 | P=0.006*     |

X^2: Chi-Square test FE_P: Fisher’s Exact test *significant at p≤0.05
Table 4: Weight of moderate and late preterm neonates

| Weight (grams) | Moderate preterm (28-<32weeks) [n=33] | Late preterm (32<37weeks) [n=27] | Significance | TKS Group [n=10] | Control Group [n=11] | Significance |
|----------------|-------------------------------------|----------------------------------|--------------|------------------|---------------------|--------------|
| At birth       | 1216.5±158.5                         | 1312.0±144.4                    | 1.750        | 1388.0±126.3     | 1298.2±189.6       | 1.143        |
| Day 3          | 1164.0±160.3                         | 1262.3±131.7                   | 1.841        | 1418.5±118.7     | 1268.8±211.9       | 1.734        |
| Day 5          | 1180.5±168.3                         | 1272.3±145.7                   | 1.611        | 1449.1±125.6     | 1300.3±225.6       | 1.734        |
| Day 7          | 1191.0±191.5                         | 1278.9±127.8                   | 1.453        | 1454.5±125.3     | 1325.3±229.1       | 1.281        |
| Day 9          | 1228.8±185.6                         | 1299.2±139.9                   | 1.168        | 1468.5±111.5     | 1341.2±229.1       | 1.155        |
| Day 11         | 1273.3±204.2                         | 1323.9±159.2                   | 0.755        | 1492.1±105.7     | 1372.7±237.6       | 1.256        |
| Day 13         | 1307.8±213.7                         | 1372.3±182.2                   | 0.896        | 1543.5±95.2      | 1387.1±248.4       | 1.633        |
| Day 15         | 1365.8±214.7                         | 1420.8±170.4                   | 0.777        | 1592.5±106.6     | 1412.1±243.1       | 1.984        |
| Day 17         | 1408.0±214.5                         | 1466.9±189.3                   | 0.806        | 1639.5±92.1      | 1471.5±248.8       | 1.507        |
| Day 19         | 1451.3±218.2                         | 1493.1±196.8                   | 0.559        | 1684.5±85.7      | 1505.6±238.2       | 1.935        |
| Day 21         | 1497.8±218.2                         | 1547.3±196.5                   | 0.641        | 1723.5±85.1      | 1510.9±266.1       | 2.034        |
| Day 23         | 1555.4±231.0                         | 1581.9±195.2                   | 0.342        | 1767.5±79.3      | 1565.9±238.6       | 2.362        |

t: Student t-test  Z: Mann Whitney test  *significant at P≤0.05

Supplementary Files

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