RESEARCH PROTOCOL

Risk factors for low physical activity levels in preschool-aged children in a densely populated urban community in Bangladesh

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Table of Contents

1. Study Summary

Title	**Risk factors for low physical activity levels in preschool-aged children in a densely populated urban community in Bangladesh**
Short Title	Preschooler Physical Activity Study (PresPA Study)
Design	Cross-sectional observational
Study Duration	1 year (August 2017 – August 2018)
Study site	Dhaka, Bangladesh
Primary Objective	To describe the physical activity levels in preschool-aged children in urban Bangladesh.
Primary Outcome	Preschooler physical activity levels (mean activity counts/15 seconds will categorize activity into: light and moderate-to-vigorous physical activity, and sedentary behavior based on predetermined cut-points).
# of Participants	60
Main Inclusion Criteria	• Child is between 34-38 months of age; • Child and primary caregiver reside in Dhaka or urban environs; • Parent/guardian provides written informed consent for study procedures.
Main Exclusion Criteria	• Child requires mobility assistance; • Child has been diagnosed with a neurological disorder affecting physical activity (e.g., Cerebral palsy) or a major chronic respiratory or cardiac disease that limits physical activity (e.g., severe asthma, congenital heart disease).
Follow-up period	N/A

Main Study Procedures	1. Accelerometry & GPS logging 2. Anthropometry 3. Blood hemoglobin concentration test 4. Home environment audit 5. Home perception questionnaires 6. Socioeconomic questionnaires

2. Title

Risk factors for low physical activity levels in preschool-aged children in a densely populated urban community in Bangladesh.

Short title: **Pres**chooler **P**hysical **A**ctivity (**PresPA**) Study

3. Protocol Version

Version 1.0 – April 13, 2017
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5. Roles and Responsibilities

5.1. Names, affiliations, and roles of protocol contributors

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6. Introduction

6.1. Background and Rationale

Physical Activity

Physical activity (PA) in childhood is a known determinant of long-term health including bone and cardiometabolic health [1,2], mental health [3,4], and the development of cognitive and motor skills [5,6]. It is especially important in the early years as it reflects the extent to which young children engage in exploratory behaviour (e.g. crawling or walking independently), a critical determinant of subsequent child development [7]. It appears that PA is protective against obesity and overweight, and PA interventions among young children have been shown to reduce body mass index (BMI), an indicator conventionally used to identify individuals as obese or overweight [8,9]. However, in young age groups, PA has been seen to play a stronger role in preventing rather than treating childhood obesity [10,11]. Childhood obesity can track into

adulthood and ultimately increase the risk of non-communicable diseases such as type 2 diabetes, gastrointestinal and hormone dependent cancers, and cardiovascular disease [12–14]. In addition, it has been seen that habits pertaining to physical activity and inactivity also track from early childhood into adulthood [15]. This is consistent with the Developmental Origins of Health and Disease theory that suggests that adult disease risk may be influenced by events that occur as early as pre-conception and into early childhood [16]. This implies that early childhood is a critical time to intervene, teach, and encourage healthy lifestyle behaviours, such as participating in PA routinely. Although there has been substantial research done in high-income countries exploring the associations between physical activity in children and rates of overweight and obesity [17,18], little is known about physical activity levels among young children living in low- and middle-income countries, or the environmental risk factors for low physical activity among children in resource-poor urban settings.

Nutrition Transition and the Double Burden

As developing countries undergo economic advancement, individuals experience a shift in their dietary habits towards diets higher in processed and prepared foods that are generally micronutrient poor and energy dense [19,20]. Factors that influence this nutrition transition include: income shifts, food prices and availability, as well as the modernization of the food industry and the influence of mass media [20]. It has been predicted that many low- and middle-income countries, including Bangladesh, will experience this nutrition transition across all income classes within the next decade [21]. This is problematic considering that in 2014, it was reported that 36% of Bangladeshi children under five years were stunted and 12% were severely stunted [22]. Since stunting is considered an indicator of malnutrition, these statistics indicate that Bangladeshi children may be subject to undernutrition and micronutrient deficiencies [23,24]. Additionally, studies have identified an increasing trend in childhood obesity and overweight, particularly in urban settings in Bangladesh [25–27]. This reveals that Bangladesh will experience an emerging nutritional 'double burden', an expression referring to the co-occurrence of high rates of both over-nutrition and undernutrition[27,28].

Current Physical Activity levels in Bangladesh

Along with the occurrence of the double burden in Bangladesh, low physical activity levels and high sedentary activities have been identified as major risk factors associated with overweight and obesity among urban school-aged children in Bangladesh [25,29]. However, these studies did not use objective assessments of physical activity such as accelerometry, a measurement technique that quantifies frequency, intensity, and duration of human movement using digital devices worn by individuals for extended periods. [30]. Furthermore, there are few data in

Bangladesh or other LMICs regarding physical activity levels of preschool-aged children. Prior research on physical activity assessment in young children has shown that objective measures using accelerometers have greater validity than parental reports of physical activity [31]. We hypothesize that in densely-populated urban settings in LMICs (e.g., Dhaka, Bangladesh), preschool-aged children cared for predominantly in the home may be at particularly high risk of low physical activity due to space constraints indoors and limited opportunities for free, safe play in the outdoors. Therefore, characteristics of the physical home environment may have dominant effects on the physical activity levels of preschool-aged children in dense urban settings. In Brazil, a cross-sectional study found significant positive associations between infant gross motor development and the amount of space inside the home, as well as number of gross-motor toys available [32]. However, few studies have addressed the determinants of physical activity levels in preschool-aged children in low- to middle-income countries and in particular, how physical activity is influenced by the home environment.

We speculate that the preschool-aged children (~3 years of age) in our study population are cared for predominantly in the home, due to the limited number of daycare centres available in Dhaka. In a 2008 report on urban planning and women's accessibility to urban facilities, it was established that there are only 13 government-facilitated and 23 private daycare facilities available in Dhaka (total population ~15 million), and the majority of them were reported to be inadequately structured and unsuitable for children [33]. In addition, it was reported that of the 41% of working mothers who participated in the survey, 94.3% rely on a maid servant to look after their children within the home, as opposed to enrolling them into daycare facilities [33]. In a more recent report from 2015, women who were interviewed reported that the major barriers to obtaining childcare in Dhaka pertains to the unavailability of daycare/preschool centres near the workplace, as well as a lack of security for toddlers, hygienic and healthy environments, and space for a playground [34]. These reports indicate that daycares are uncommon in this setting, and that children in Dhaka, especially preschool-aged children, spend their days within the home.

Influence of Iron Deficiency on Physical Activity

In addition to indoor and outdoor aspects of the built environment, undernutrition itself may adversely affect young children's physical activity levels,[35]. Prior work has shown that undernourished infants, specifically those with iron deficiency, have lower levels of physical activity that may contribute to functional isolation and developmental delays [36]. In an early study by Lozoff and colleagues, they found that iron-deficient anemic infants had lower endurance levels, excess fatigability, and showed indications of being less active [36]. It was also seen in a subset of infants at risk of micronutrient deficiencies in rural Bangladesh that

administering weekly supplements of iron and zinc protected children from a decline in motor development and exploration [37]. The effect sizes of children who were administered a supplement containing a 16 micronutrient mix that included iron and zinc were similar to those that just received zinc and iron, indicating that other micronutrients may not be as important in infant motor development as iron and zinc alone [37]. Additionally, a case-control study conducted in Indonesia found that when compared to the non-anemic, non-iron deficient control children, anemic, iron-deficient infants who received iron supplements developed motor skills more quickly and displayed greater physical activity [38]. Although these children may be experiencing motor advancements due to nutrient "catch-up", this still indicates that iron-deficiency anemia may be a potential risk factor for low physical activity levels in young children. Considering the high prevalence of undernutrition and anemia in young children in Bangladesh, these may be important factors to consider when evaluating physical activity levels within this population.

Study Rationale

As the double burden of nutrition emerges in Bangladesh and other low- to middle-income countries, it is imperative to develop strategies to mitigate the adverse effects associated with both undernutrition and obesity. Identifying levels of physical activity among children is a requisite step to developing and targeting interventions to promote physical activity among young populations. Furthermore, exploring the risk factors associated with physical activity levels in young children can help better inform policy makers surrounding a public health intervention.

7. Objectives

Goals

The overall goal of our research is to generate new knowledge regarding the nutritional and environmental determinants of physical activity in young children living in a densely populated urban community in Bangladesh.

Primary Objectives

The specific objectives of this study are to:

1. Describe physical activity levels in a sample of preschool-aged children in an inner-city community in Dhaka, Bangladesh,
2. Estimate the associations between characteristics of the physical environment of the home (total area of available floor space inside of the home, number and presence of

physical hazards, and the number of gross motor activity-oriented items present) and the physical activity levels of preschoolers in Dhaka, and
3. Estimate the associations between hemoglobin concentration (Hb) and preschooler physical activity level.
4. Describe sleep quantity and quality in the sample of preschool-aged children in urban Bangladesh

We hypothesize that low levels of preschooler physical activity are associated with a lack of play-oriented physical attributes (i.e., total area of indoor floor space, presence and count of unsafe physical hazards, and presence and count of stationary and portable gross motor activity-oriented items) within the homes in urban Bangladesh. We also speculate that low Hb may be associated with low physical activity levels in this population.

8. Setting and Participants

We propose to conduct a cross-sectional observational study of preschool-aged children between 34-38 months of age (n=60) selected from the ongoing Maternal Vitamin D for Infant Growth (MDIG) trial cohort in Dhaka, Bangladesh. The study will take place in urban Dhaka, Bangladesh. We will enroll a minimum of 60 participants with complete data sets, but will recruit up to 90 participants until this enrolment target is reached.

8.1. Inclusion Criteria

- Child is 34-38 months of age;
- Child and primary caregiver reside in Dhaka or urban environs;
- Parent/guardian provides written informed consent for study procedures.

8.2. Exclusion Criteria

- Child requires mobility assistance and/or has been diagnosed with a major neurological or orthopedic condition or disorder affecting physical activity (e.g., cerebral palsy, club foot, etc.).
- Major physician-diagnosed chronic respiratory or cardiac disease that limits physical activity (e.g., severe asthma, currently taking medication for symptomatic congenital heart disease, etc.).

9. Methodology

9.1. Study Procedures

The majority of data collection will take place during the initial home visit, including collection of anthropometric measures and a home built environment audit. Accelerometers and GPS loggers will be provided during the initial home visit. The participant will then take part in 7 days of accelerometry and GPS logging data collection consecutive or non-consecutively over a 14 day time period. A final visit will take place at the end of the data collection period where data surrounding the perception of the home environment and household food security will be collected, as well as participant hemoglobin concentration will be measured. This final visit will occur at the clinic where the study physician and/or paramedics can safely measure hemoglobin collection. We will ask the caregiver of the participant to bring the accelerometers and GPS loggers to the clinic where they will be collected for analysis by study personnel.

9.2. Recruitment

Participants within the Maternal Vitamin D for Infant Growth (MDIG) trial (NCT01924013) cohort registry who have a) expressed interest in participating in future sub-studies, and b) have children within the target age range, will be contacted. Participants will be contacted prior to their anticipated study visit. A contact list will be updated each week to prospectively recruit participants in advance or during the age window in which children are eligible for enrolment. Each list will begin with the eldest children to reduce the number of missed opportunities to enroll children before they exit the eligible age window. The catchment area will include the following neighbourhoods in urban Dhaka: Kamrangir char, Azimpur, Lalbag, and Hazariba

Local study personnel will make initial contact via telephone call. If a participant does not answer the phone call and therefore has not declined participation, they will continue to be contacted 2 additional times overall until a) the child is no longer within the eligible age range, b) contact is made and the participant enrolls in the study, or c) contact is made and the participant declines joining the study. Study personnel will follow the Screening Script to ask each caregiver a series of questions to determine if their child is eligible (Screening Form). Once a participant is successfully enrolled, ages out of the eligible age range or declines participating in the study, they will be removed from subsequent contact lists.

If a participant meets all inclusion criteria and does not meet any exclusion criteria, an initial home visit will be scheduled at the convenience of the participant and their caregiver. Informed consent and study enrolment will occur at the beginning of the Initial Home Visit, prior to any

data collection procedures.

9.3. Data Collection

9.3.1. Initial home visit: Consent (caregiver)

The home visit will begin by having the participant's caregiver review and sign the PresPA Study Consent Form (A); this is necessary to obtain parental permission. In Bangladesh, enrolment is permissible with only one legal guardian providing consent. A verbal reading of the consent form will be provided if the caregiver of the participant is illiterate. Once the participant's caregiver has signed or thumb stamped the consent form, data collection can begin.

9.3.2. Initial home visit: Socioeconomic questionnaire (caregiver)

A primary caregiver (usually mother) of the preschooler participants will be asked to participate in the collection of socioeconomic data, including education level and questions pertaining to wealth (which will be used to develop the overall wealth index; Form 1). Due to the low literacy rates within the target population, this will be done in a structured interview format.

9.3.3. Initial home visit: Anthropometric measures (child and caregiver)

Next, the preschooler participant and their caregiver will take part in anthropometric measures (Form 2). Using a portable lengthboard/stadiometer (ShorrBoard™, Shorr Productions, Olney, MD, USA), duplicate measures of height will be collected from both child and mother. Child height will be collected during the initial home visit, whereas maternal height will be collected during the final clinic visit using a stadiometer (Seca-217, Seca Corp., Hamburg, Germany). If there is a discrepancy of 1 cm between the first and second measure, another set of measurements will be collected. If a child does not cooperate in standing during this measure, study personnel will be instructed to lay the lengthboard/stadiometer on the ground to obtain a recumbent length measurement. Height measurements obtained from a recumbent length measurement will be adjusted for by subtracting 0.7 cm before plotting on the World Health Organization (WHO) growth curves [39].

Weight measurements will be collected using a portable scale (Seca-874, Seca Scale Corp., Hamburg, Germany). Duplicate measures of weight will be collected from both child and mother. If there is a discrepancy of 50 g between the first and second measure, another set of measurements will be collected. If a child is unable to stand still during the weight measurement, their caregiver can be measured while holding the participant. The caregiver's

body weight will then be subtracted from the combined measure (participant and caregiver) to obtain the participant's body weight.

Preschooler participant and caregiver waist circumference will be collected next using a non-stretch, compression spring tape measure (Gulick II measuring tape, Country Technology Inc., Gays Mills, WI, USA). As per the recommended protocol from Statistics Canada, NHANES, and the Canadian Health Measures Survey, waist circumference will be measured at the top of the iliac crest [40]. These measures will be taken in duplicate; if there is a discrepancy of more than 0.5 cm between the first and second measure, a second set of measurements will be collected. Measurements will be taken at the end of a normal expiration (after expiration, before inspiration).

Lastly, we will collect bioelectrical impedance (BIA) measures from both child and caregiver using the Quantum II – Body Composition Analyzer™ (RJL Systems, Clinton Township, MI, USA). This single-frequency, portable device utilizes the hand-to-foot technique, in which 4 electrodes are attached to the body, two on the hand and two on the foot, while lying supine. BIA estimates total body water (TBW) by sending a low voltage electric current through the body between the electrodes, in which resistance and reactance of the current are measured. The current runs quickly through water present in hydrated muscle tissue (fat free mass; FFM), but will experience resistance in fat mass (FM). Therefore, the more FFM, the more body water one has and the less resistance the current will experience. Resistance and reactance values collected from this device will then be inputted into a validated equation to properly convert TBW to FFM. Participants must be lying supine and relatively still in order to obtain precise measures. If a child does not follow these procedures, their caregiver can lay supine with them, so long as they do not touch the child's body; soothing the child by caressing their head is acceptable, as it will not affect the precision of the measurements (since the current runs only through the hand, up the arm, down the torso and towards the foot on the right side; between electrodes). If an individual does not comply and refuses to lie still, fluctuation in resistance and reactance values will arise. Any protocol deviations, including lack of cooperation by the participants and/or their caregiver during this measure, will be recorded (seen in Form 2) and the appropriate measures will be excluded from analyses.

For the preschool-aged participants, we will use an equation validated against ^{18}O dilution in well and malnourished young children developed by Fjeld and colleagues [41]:

$$TBW = 0.76 + 0.18H^2/R_{50} + 0.39W$$

where TBW is in kg; H, height in cm; W, weight in kg; R, resistance in ohms at 50 KHz. This equation was developed for both boys and girls and therefore is not sex-specific. Although this equation was developed and validated in a population from Peru [41], it was validated again in a population of preschool-aged children in urban India [32]. They found that the FFM values that this equation predicted was in good agreement with those derived from TBW measured by D_2O dilution [42,43]. This equation was chosen for the PresPA study due similar characteristics between urban Indian preschoolers and urban Bangladeshi preschoolers and due to the fact that no equations validated in Bangladeshi children exist to date.

To determine FM from TBW, the hydration constants developed by Fomon and colleagues will be used, as they remain the reference standards for preschoolers: 0.775 (boys, 3 years), 0.779 (girls, 3 years) [44]. TBW will be divided by these factors to calculate FFM (kg). FM (kg) is then determined by calculating the difference between total body weight (kg) and FFM (kg).

To evaluate the body composition of the female primary caregivers (usually mothers) of the preschoolers, we will use an equation validated in Bangladeshi postpartum women against $^2H_20$ dilution criterion method:

$$TBW = 4.297 + 0.190*W + 0.349*H^2/R_{50}$$

where TBW is in kg; H, height in cm; W, weight in kg; R_{50}, resistance in ohms at 50kHz [45]. FFM (kg) can then be determined by dividing TBW by 0.732, assuming that this population's FFM has a hydration factor of 0.732 [45]. FM (kg) is then determined by calculating the difference between total body weight (kg) and FFM (kg).

BIA measures will be collected in duplicate. If the second resistance and/or reactance value has a discrepancy of greater than 5% from the first value taken, a second set of measures will be collected. Participants and their caregivers will be asked to remove any metal material (i.e., belt clasps, underwire bras, etc.), as this will disrupt the pathway of the electrical current. Additionally, caregivers will be asked if they have a pacemaker (Form 2). Those who agree to having a pacemaker will automatically be opted out of providing BIA measures, as the current may interrupt its functionality. Child participants will be screened for cardiac conditions, including having a pacemaker, prior to the initial visit during the recruitment process (Screening Form).

Participants and their caregivers can choose to opt out of any of the anthropometric measures without penalty. Mothers who are pregnant will be automatically opted out of waist circumference and BIA measures.

If any anthropometric measures are not collected during the initial home visit (e.g., measuring device is not functioning properly, participant does not co-operate, etc.), measures may instead be collected during the final clinic visit.

9.3.4. Initial home visit: Home built environment audit (caregiver)

Before the home environment audit can begin, study personnel will obtain permission from a full-time resident of the household for which measurements are requested. The PresPA Study Consent Form (B) allows for photography to be used to capture the built physical environment of the home. Once consent is provided, the home audit can begin. If the owner of the home refuses to sign Consent Form (B), the home built environment audit will still proceed; however, no photography will be conducted in the home. The participant and their caregiver are still eligible to participate in all other study procedures, with the exception of the collection of photographs.

Once consent has been given, study personnel will ask the caregiver if there are any restricted areas within the indoor home environment where their child is not allowed access. These areas will not be included in total available floor space (m^2) of the indoor space. The study personnel will then draw the layout of the indoor space (Form 3). Then, using a laser measuring device (Disto^{TM} D1, Leica Geosystems, Switzerland), the study personnel will measure the dimensions of the space, collecting measures in duplicate. They will include any furniture that is greater than 1 m^2, including each piece of furniture in the drawing of layout, as well as measuring the dimensions of furniture in duplicate using a standard tape measure. Study personnel will not include any beds or sofas in the drawings, as they will not be subtracted from the available indoor floor space (since it is common for children to use beds or sofas as a play space in Dhaka). They will also be asked to take at least 2 photographs of each room within the home, consent permitting, to accompany the area measurements. These pictures will not include any individuals and will strictly capture the physical built environment of the indoor space.

Next, the study personnel will ask about any outdoor spaces that are accessible to the child. Only outdoor spaces that have clear perimeters (e.g., fenced in backyard, railings or walls surrounding a rooftop, railings surrounding a balcony) will be measured using the laser-measuring device. If the laser does not operate in the outdoor environment (e.g., weather conditions do not permit the proper lighting standards necessary to obtain a reading), a standard tape measure will be used. Open streets, alleyways, large parks or green spaces, or outdoor spaces that do not have a clear perimeter will not be measured; however, they will be documented and physical characteristics will be described by study personnel in the

appropriate sections of Form 3. Study personnel will be asked to photograph all outdoor spaces, consent permitting, as long as they do not include any individuals within the pictures.

Once the indoor, and if applicable, outdoor area has been measured, the study personnel will assess the home environment for physical hazards. Using the audit provided (Form 3), they will assess the presence and safety surrounding stairwells, accessibility to stovetops and open sewage drains, as well as the presence of unfinished construction and common household hazards (e.g., boti, a common household cutting device in Bangladesh). A check list will be provided to the caregiver at the final visit which will indicate if any of the 5 hazards that we are assessing are present in their home. In addition, we will provide them with strategies to increase safety of their children (e.g., put up railing around stairwells, keep boti in a space that isn't accessible to the child, etc.).

Finally, study personnel will take an inventory of any gross-motor activity-facilitating items within the home (Form 3). They will ask the caregiver to show/bring them items with which they allow their child to play on/with. Study personnel will then document each item on the audit form (From 3) and take photographs of each item (up to 10 items). These photographs will not include individuals, but will strictly capture the gross-motor activity-facilitating items present in the home. During data input, the student investigator will evaluate these items and place them into specific categories: fine-motor activity item, stationary/fixed gross-motor activity item, portable gross-motor activity item (ball play equipment, push/pull equipment, riding equipment, rocking and twisting equipment) [46,47].

9.3.5. Accelerometry & Global Positioning System (GPS) logging (child)

At the end of the initial home visit, the study personnel will provide the participant with their activity belt. The belt will consist of a tri-axial accelerometer (ActiGraph GT3X-BT, ActiGraph Corp., Pensacola, FL, USA) that will be positioned at the right hip, and a passive GPS logger (iTrail H6000, KJB Security Products Inc., TN, USA) that will be either positioned at the left hip of the child, or on the arm of the caregiver. The placement of the GPS logging device will depend on the participant's comfortability with wearing the device on the activity belt, otherwise it is acceptable for the caregiver to wear it on their person. Both the accelerometer and GPS logger will be charged and initialized prior to the home visit. The study personnel will then provide the caregiver with instructions on how the belt is positioned on the child and explain that the child is to wear the belt 24 hours each day. The belt should only be removed during periods of bathing and swimming. As mentioned above, to improve child comfort during the day or while sleeping, the caregiver may remove the GPS device from the pouch attached to the belt at anytime and wear it on their person (a separate, additional belt will be provided to the

caregiver during the initial home visit), so long as they are with their children at all times during the day. Caregivers can remove the belt while sleeping if they find it uncomfortable, but must reattach it each morning.

The accelerometer will objectively measure all movement of the device-wearer continuously. The device is to be worn continuously for 7 days; however, the child can wear the device for 7 days non-consecutively within a 14-day time period if the caregiver identifies that the child is travelling away from home, is ill (e.g., illness that parent perceives as greatly affecting PA, such as fever, major injury, etc.), bedridden, or hospitalized. Accelerometry and GPS data will not be included in analyses during those specified days. This will continue until the caregiver indicates that the participant has returned home from travelling or is no longer ill, bedridden, or hospitalized, when accelerometry collection can resume. Study personnel will gather this information via the daily telephone calls with the participant's caregiver (section 9.3.6).

The accelerometer device will be set to collect data at 30 Hz (i.e., it will take readings 30 times per second) to ensure high-quality data. Data can only be viewed after the device has been collected from the participant and uploaded into the software program (ActiLife v10). Activity is measured in counts, and is analyzed over specified time periods. Once the number of counts has been analyzed over a specified time period, the data point is then referred to as an 'epoch'. Data will be analyzed in 15 second epochs (counts per 15 seconds; cp15s).

The GPS logger will objectively measure the coordinates of each location that the participant travels. As with the accelerometer, the GPS logger device is to be worn continuously for 7 days, or non-consecutively within a 14-day time period. The collection of location data will occur simultaneous to the collection of accelerometry data. To ensure quality location data, the device will be set to take readings every 15 seconds, as this will directly correspond to the 15 second epochs calculated from the processed accelerometry data. This data will be used in combination with the accelerometry data to gain insight on the location of preschooler activity (e.g., home, park, relative's home, street, etc.). Similarly, as with the accelerometry data, data collected from the GPS logger can only be viewed after the device has been collected from the participant and downloaded into the software program (iTrail GPS Mapping and Trip Analysis Software). This passive GPS device does not offer remote real-time tracking; therefore, it cannot provide real-time location data and data can only be collected once the device has been returned.

The data collected from the GPS logger in combination with the accelerometry data will substantially increase the measured accuracy, sensitivity and objectivity of an individual's exposure to environments while they engage in active behaviour [48]. Together, the will

objectively, and accurately, measure the types of environments that individuals are exposed to, the duration they were there for, and what kind of active behaviours they participated in [48].

The combination of accelerometers and GPS logging devices have been used in a few studies that measure the risk factors surround PA of preschool-aged children specifically [49–51]. Each of these studies utilizes an activity belt system on which we have modeled our design (e.g., accelerometer positioned on the right hip; GPS logger positioned on the left hip).

9.3.6. Daily phone calls (caregiver)

Every afternoon, study personnel will call the caregiver and go through a daily recall of (a) what time the participant woke up in the morning, (b) if, and when, the activity belt was removed during the participant's waking hours, (c) what time the participant went to sleep, and (d) if the participant left the home during the day (including location and duration away from home). This information will be documented in the Accelerometry & GPS Log each day. If a participant's caregiver cannot be reached on any particular day, study personnel will attempt to call 2 additional times that day. If contact has not been made, study personnel will contact the participant's caregiver the next morning and ask them to recall the previous day, then call again in the afternoon to recall the current day; a normal calling schedule will resume. If contact has not been made within 3 days, which has not been previously agreed upon between the participant's caregiver and study personnel (e.g., the participant is travelling, sick, etc.), the participant will be removed from the study and study personnel will visit the participant's home to collect the activity belt.

9.3.7. Final visit: Home perception questionnaire (caregiver)

Once the caregiver has indicated that the child has completed their 7 days of wearing the activity belt, study personnel will schedule a clinic visit at the convenience of the participant and their caregiver. If the participant is unable to travel to the clinic, a home visit can be arranged instead. During the final visit, the participant's caregivers will participate in the Home Perception Questionnaire (Form 4) in the form of an interview conducted by study personnel. This questionnaire consists of questions pertaining to the caregiver's perception of the adequacy of facilitating physical activity, safety, and cleanliness inside and outside of the home. This questionnaire will also capture the known physical activity risk factor: screen time [52,53]. In addition, this questionnaire will collect information on food security, a potential risk factor for low hemoglobin levels, and iron supplement usage. The Home Perception Questionnaire will be conducted during the final visit as opposed to the initial visit in order to avoid influencing the caregiver and effect how the participant wears the activity belt during the accelerometry data collection period.

The caregivers of the participants can opt out of the home perception questionnaire, if they choose to, without penalty.
In addition, child participants within the study will have the opportunity to participate in a general physical checkup during the final clinic visit by our study physician. Participation in the general physical is not mandatory, but is offered as a health incentive to participate in the final visit of the study.

9.3.8. Final visit: Hemoglobin concentration collection (child)

Hemoglobin concentration will be collected from the child participant one time during the final visit in the clinic (or the home if needed). The study physician or paramedics will ensure that the child is in a seated position before proceeding to clean the child's left index finger with an alcohol wipe. They will proceed to prick participant's left index finger with a spring-loaded needle. Squeezing from the base of the finger to the tip, the research personnel with then encourage blood to flow out of the participant's finger. In order to prevent an inaccurate hemoglobin reading, the first drop of blood will be wiped away with the alcohol swap and the squeezing process will repeat. Once a second drop of blood emerges from the participant's finger, a hemoglobinometer (HemoCue, Helsingborg, Sweden) will be used to collect the drop of blood and provide a hemoglobin concentration reading (to be documented in Form 4).

If an individual has a reading <110 g/L, therefore indicating mild anemia, the study physician will be informed. The individual will then be referred to the local clinic to be evaluated by a physician. The Physician Referral Log will keep track of all anemic participants, date of referral, whether the participant accepted or declined the referral, date of physician appointment, if the participant attended appointment, treatment dosage, and reimbursement amount. If iron supplements are prescribed, we will reimburse the participant the amount spend on the treatment. Detailed instructions will also be provided to the caregiver on the use and safe storage of the supplements.

If an individual has a reading <90 g/L, therefore indicating moderate or severe anemia, the study physician will be informed. As stated above, the individual will then be referred to the local clinic to be evaluated by a physician. All cases of anemia will be documented on Form 4 and will be brought to the supervisor's attention, then included in the Physician Referral Log. The Student Investigator will ensure that the study physician follows up with these participants, and provides reimbursement for any iron supplements prescribed for treatment.

This measure was designed to be collected post accelerometry collection in order to prevent any potential influence iron supplements may have on the participant's physical activity, specifically for those identified with anemia.

An individual has the right to refuse participating in the hemoglobin concentration collection, and will not be penalized for declining this aspect of the study. They will not be included in analyses involving individual hemoglobin concentrations.

9.3.9. Final visit: Collection of accelerometer and GPS logger

Prior to the final visit, study personnel will remind the caregivers of the participants to bring the activity belt(s) to the clinic for their appointment. They will then collect the activity belts (including the accelerometer and GPS logger) from the participants. Data from both devices will then be downloaded and analyzed accordingly (see Section 9.3.5). Once the activity belt has been received, compensation will be provided to the participant's caregiver for their time, travel, and participation in the study. As compensation for the participants for participating in the study, we will then be translating their PA data into a child-friendly, graphics-based activity report that each participant will receive post-analysis.

This activity report will be explained to the parents. Based on the report, study personnel will either inform the caregiver to either encourage child physical activity levels or maintain current levels for optimal child health.

9.4. Expected study duration (for each participant)

The caregivers of the participants will be contacted for recruitment prior to their anticipated home visit. Study personnel will arrange a convenient time for the participant and their caregiver to conduct the initial home visit.

Each participant will have a minimum study duration of 5 days and a maximum of 15 days. This will include: the initial home visit on Day 1; between 7-14 consecutive or non-consecutive days of wearing the activity belt (accelerometry and GPS logging); and the final visit in the clinic, which will occur once the participant has worn the activity belt for at least 4 days.

The initial home visit is expected to last between 1-2 hours. Each daily phone call is expecting to be between 5-15 minutes each day. The final home visit is expected to last between 1-1.5 hours minutes.

9.5. Participant removal criteria

Participants will be removed from the study if any of the following occur:

1. Participant refuses to participate in accelerometry collection (as this is the primary outcome measure of the study);
2. Contact with the participant's caregiver has not been made in over 3 days;
3. Caregiver withdraws participant consent.

9.6. Sample size and power calculations

Given 126 screened children, we expect to enroll up to 75 children in the study. For our primary objective, we will need 60 enrolled children with complete accelerometric data to be able to describe the mean counts/15 sec (cp15s) with a margin of error of ±2.45 cp15s, assuming a standard deviation of 9.7 cp15s [54].

10. Analysis

10.1. Outcome measure

The primary measure of physical activity will be a continuous variable that represents the average total physical activity level of each child, expressed as a mean counts per 15 seconds (cp15s) averaged over all of the child's eligible wear time on all days that meet criteria for inclusion.

Due to the young age of the participants, compliance for a full 7 days may prove to be difficult; therefore we will use 3 days of data as the minimum number of valid days per child [31]. If a child has more than 7 days of eligible wear time, we will include all additional days with sufficient wear time.

Accelerometry data is collected at 30 Hz, meaning that data measures are collected 30 times per second. Activity counts collected within the 30 measures/second are averaged to provide an activity count for that particular second. We will be analyzing the data in sections of 15 seconds, therefore we will be averaging activity counts within 15 second intervals (average 15 data points of activity counts). These 15 second epochs (cp15s intervals that have been analyzed) will be used to analyze physical activity levels by categorizing them into sedentary behaviour (SED; sitting, laying down, etc.), light physical activity (LPA; walking, crawling, etc.), and moderate-to-vigorous physical activity (MVPA; running, jumping, etc.) using the cut-points chosen [55].

We will use the cut-points established by Trost and colleagues: ≤48 cp15s for sedentary behaviour (SED), 49-418 cp15s for light physical activity (LPA), and >418 cp15s for moderate-to-vigorous physical activity (MVPA) [55]. These cut-points were chosen because they were derived from direct observation of a sample of toddler and preschool-aged children while wearing the ActiGraph GT1M accelerometer [55]. Videos of the children playing were coded using a modified version of the Children's Activity Rating Scale before receiver operating characteristic curve analyses were conducted to determine the corresponding cut-points to the 3 categories of interest: SED, LPA, and MVPA [55]. These results were found to exhibit excellent classification accuracy [55]. They were then cross-validated against pre-existing cut-points that are established for toddlers and preschoolers, which were not found to be significantly different than the pre-established cut points established by Pate and colleagues [56] and those established by National Health and Nutrition Examination Survey [55].

We will then be able to calculate total time (minutes) as well as proportion of time (%) spent in each activity category (SED, LPA, MVPA). The overall mean cp15s for each child will be based on all 15-second epochs that occurred during eligible wear periods from the time that the caregiver indicated the child woke up until the time that the caregiver indicated the child went to sleep at. If this data is missing, we will use eligible wear periods between 8am to 8pm. This mean will exclude non-wear periods as defined above and when the accelerometer was not being worn by the child (based on daily logs collected from the caregivers via afternoon telephone call; Section 9.3.7.).

For each child, we will calculate the mean number of minutes per day and the proportions of the child's total eligible wear time that are classified as SED, LPA and MVPA. We will also determine the proportion of days on which each child reached or exceeded 180 minutes of activity classified as LPA or MVPA, and the proportion of days on which each child reached or exceeded 60 minutes of activity classified as MVPA. These cut-offs are based on the Canadian preschooler physical activity guidelines established by Tremblay and colleagues [57], as there are no Bangladeshi standards available at present. If Bangladeshi guidelines are released before data analysis and reporting is completed, we will also apply those standards in our analyses.

For each child, we will establish the following parameters related to accelerometry:

- Total duration (minutes) of the potential data collection period on all days on which the accelerometer was instructed to be worn;
- Total eligible wear time (minutes), including data collected while the child was awake (from the time that their caregiver indicated that they woke up until the time that they went to sleep). If this data is not provided, total eligible wear time will be measured between 8am and 8pm. Non-wear periods will be excluded based on the following

criteria: ≥20 minutes of 0 cp15s allowing for up to 2 minutes of 0-100 cp15s in that period [31]. A given day will be considered eligible for inclusion if there are at least 10 hours/day (600 min) between the indicated time period that the child was awake at, or between 8am to 8pm of wear time for those with missing data, after excluding non-wear periods;

- Total indoor eligible wear time (minutes; excluding non-wear periods as previously defined), based on maternal report;
- Total eligible wear time in or around the home (minutes; excluding non-wear periods as previously defined), based on combination of maternal report and GPS logging. "Around the home" will be defined as occurring in the same building or property of the home (e.g., rooftop, neighbouring apartment in the same building, common areas of the apartment building, private courtyard areas that are on the same property as the home in which the child resides).

For purposes of secondary and sensitivity analyses, we will also estimate for each child:

- Mean cp15s for indoor activity
- Mean cp15s for activity in or around the home
- Mean cp15s on a day that is typically a work day (Sunday through Thursday)
- Mean cp15s on a Friday and/or Saturday (weekend)

For each of the above measures, we will also classify the activity in terms of average daily minutes and proportion of total time spent in SED state, LPA state, and MVPA state. In sensitivity analyses, we will compare the average daily minutes and proportion of total time spend in each activity category across different cut-off points that have been established by various research groups:

- Proportion of time spent in activity categories established by Pate and colleagues (SED: ≤199 cp15s; LPA: 200-419 cp15s; MVPA≥420 cp15s)[56]
- Proportion of time spent in activity categories established by Sirard and colleagues (SED: ≤301 cp15s; LPA: 302-614 cp15s; MVPA ≥615 cp15s)[58]Proportion of time spent in activity categories established by Van Cauwenberge and colleagues (SED: ≤372 cp15s; LPA: 373-584 cp15s; MVPA ≥585 cp15s)[59]

To address aim 4, we will analyze sleep quality and quantity directly on the nighttime accelerometry wear periods. Sleep data will be measured in 60-second epochs. This is obtained from the same accelerometry file that the activity data is derived from; however, data will be re-integrated into a 60-second epoch file (analyzes number of activity counts in every 60-second period).

Using the manufacturer's add-on to the ActiLife software (ActiLife 6 Full sleep Features Upgrade), we will be able to detect the number of sleep periods as well as the duration and quality of the sleep periods within the accelerometry file for each participant. The software will also detect and describe:

- Time in bed/out of bed (am/pm); duration in bed (hours; min)
- Sleep onset (when sleep commenced; am/pm)
- Total Sleep Time (TST; hours, min)
- Wake After Sleep Onset (WASO; describes how many minutes after sleep onset the participant woke up; min)
- Awakenings (number of awakenings during sleep onset)
- Average awakenings (average duration of awakenings during sleep onset; min)
- Total activity counts during sleep period (cp60s)
- Movement Index (MI)
- Fragmentation Index (FI)
- Sleep Fragmentation Index (SFI)
- Sleep Efficiency (%)

For descriptive statistics, these measures will be averaged over the number of eligible nighttime wear periods for each participant. In extended analyses, each eligible nighttime wear period will be considered as the unit of analysis.

The accelerometry data will either be processed using the software supplied by the manufacturer (ActiLife ver6) or using a published code to analyze raw accelerometer data [60].

10.2. Exposure variables

1) Total area of available floor space inside the home (m^2)
2) Frequency that child plays outdoors
3) Total number of physical hazards in the home (presence of open stairwells, stove height)
4) Total number of fine-motor activity items available to the child
5) Presence of gross-motor activity items available to the child across each category (e.g., fixed, portable: ball equipment, push/pull equipment, riding equipment, rocking and twisting equipment)
6) Hemoglobin concentration (g/L).

Descriptions and derivations of each of these variables can be found in Table 1 (Appendix).

10.3. Other covariates

Other covariates will include: preschooler sex, preschooler anthropometric measures (e.g., height-for-age z-score, BMI-for-age z-score, waist circumference, and body fat percentage), maternal anthropometric measures (e.g., BMI, waist circumference, and body fat percentage), maternal and paternal socio-economic factors (educational level and household assets), and household food security status. Descriptions and derivations of each of these variables can be found in Table 2 (continuous variables) and Table 3 (categorical variables) (Appendix).

10.4. Statistical Analysis

10.4.1. Primary Analysis

Statistical analyses will be performed using STATA (College Station, TX). Data from Forms 1, 2, 3, 4, and the Accelerometry and GPS Log will be double entered in real time independently by research personnel. Data forms will be de-identified, such that only a participant ID number will be used to identify participants. Any discrepancies between data enterers will be flagged and reconciled by the student investigator. Data reconciliation will be conducted by the student investigator and will consist of reviewing the original hardcopy of the document and/or discussing the measure with the research assistant who had originally recorded it. Data review will consist of creating histograms, boxplots, and normal quantile-quantile (Q-Q) plots of each continuous variable to identify outliers and potentially implausible data.

Each day, the student investigator will assess the hardcopies of the data collection forms for missing data. In the case that data that can be collected over the telephone is missing (e.g., socioeconomic information, age, home perception questionnaire measures, etc.), if the missing data is flagged within 60 days of the date of initial collection, study personnel will reach out to the participant and their caregiver via telephone call to collect the measure of interest. If the missing data is flagged outside of the 60-day window of re-collection, the participant will be automatically opted out of that particular measure. In the case that data that must be collected in person is missing (e.g., body composition measures, dimensions of the home environment, hemoglobin concentration, etc.), if the missing data is flagged within 21 days of the date of initial collection, study personnel will reach out to the participant and their caregiver to schedule another home visit to collect the measure of interest (e.g., if measures are missing from the initial home visit, they may be collected in person during the final visit). In the case that the participant or their caregiver has opted out of a particular measure (e.g., hemoglobin concentration), they will not be included in analyses that involve that measure of interest.

Objective #1: *Describe physical activity levels of the sample of preschoolers in urban Dhaka.*

To address our first objective, we will first create a histogram of the distribution of the data to assess the spread and shape of the frequency distribution of each of the categories of all of the physical activity data (average cp15s across all days for each participant). The number of bins within the histogram will be determined using the equation $1 + 3.3\log_{10}(n)$. A boxplot will then be created to assess the spread, as measured by the median; interquartile range (IQR); range of observations; presence of outliers; and provide additional information about the shape of the data. Finally, a Q-Q plot will be created to compare the frequency distribution of the data to a normal distribution. Using the shape and direction of the curvature, we will assess any deviation from normality.

We will estimate the mean and 95% confidence interval of cp15s for total physical activity of the sample of children, overall and stratified into the following groups:

- Boys and girls
- BMI categories (underweight, normal, overweight and obese)
- Household food security status (food secure, moderately food insecure; severely food insecure)
- Anemic and non-anemic

We will then summarize the distributions of the average amount (# of minutes) and proportions of wear time spent engaging in SED, LPA, and MVPA, over the measurement period. Distributions of each time spent in each level of activity will be shown graphically (histograms, kernel density plots) and summarized using conventional descriptive statistics: mean and standard deviation if normally distributed, or median and interquartile range if not normally distributed.

We will then estimate the frequency of children who met Canadian physical activity recommendations on any day, and the average number of days on which children met the recommendations (180 minutes of activity classified as LPA or MVPA, working towards 60 minutes of activity classified as MVPA)[57]. Bangladeshi recommended guidelines for preschool-aged children will be used if they are released before statistical analyses begin. These data will be compared to published results in similar international, preschooler populations.

The above analyses will be repeated using data restricted to indoor wear time, in and around the household wear time, on weekdays, and on weekends.

In addition, we will be using the GPS logging data to create 'heat maps' for each of the participants' physical activity locations. This will first be done at the individual level using kernel density estimation and smoothing. These individual 'heat maps' can be combined in a master map that will create a visual piece that can aid in describing study findings. We will then use the heat maps for each individual to estimate the average diameter of the 'sphere' of activity (km) for the entire study population.

Objective #2: *Estimate the associations between characteristics of the physical environment of the home environment (total area of available floor space inside of the home; frequency that child plays outdoors; number of physical hazards present within the home; number of stationary gross-motor activity-facilitating items present; and number of portable gross-motor activity-facilitating items present) and preschooler activity level.*

To address our second objective, we will first graph each of the variables of interest to assess the data for outliers or implausible data. We will generate histograms, box-plots, and Q-Q plots to assess the following continuous variable:

- Total area of available floor space inside of the home (m^2); Height of stove inside the home (m);

We will generate medians and IQRs for the following discrete, continuous variables:

- Open stairwells in the home (no railing);
- Presence of fine-motor activity-facilitating items;
- Presence of gross-motor activity-facilitating items (for each category).

These discrete, continuous variables may be categorized and treated as categorical variables depending on the data. To assess the linearity between these continuous exposures and outcome measure (using each parameter defined in Section 10.1.), we will create scatterplots and generate the correlation coefficient (Pearson correlation) for each relationship. A locally weighted scatterplot smoother (LOWESS) technique will also be used to check for departures from linearity. If the association between the exposure and the outcome is linear, we will proceed with the linear regression. However, if the association is non-linear, we will proceed with a linear spline model.

Descriptive statistics:

We will calculate the mean and standard deviation of the following continuous measures:

- Area of available floor space indoors;
- Population density of indoor space (number of people dwelling in the home/total available area of indoor floor space);
- Height of stairwell railings (of those that had a railings); and

- Height of stove (of those who had stoves)

We will estimate the median and interquartile range of the following discrete measures:

- Number of rooms within the homes;
- Number of people who live in the home

We will generate tabular descriptions (frequencies and percents) of the following measures:

- Homes that had open stairwells (no railings);
- Homes that that had minor (<1m^2) unfinished construction (of those who had unfinished construction);
- Those who had major (>1m^2) unfinished construction (of those who had unfinished construction);
- Building materials that the activity-facilitating items in the home are made of (e.g., clay, plastic, metal, wood, cloth, other).

We will use a mixed linear model with a random child-specific intercept. This is due to the repeated measures collected over the 7-day period for each participant. The physical activity data will be analyzed in activity counts per 15 second epochs, however to create multiple observations, we will nest the data by averaging the epochs over each of the 7 days to create 7 separate observations in each of the participant's datasets. This method was chosen since it will appropriately model the means and standard errors as well as properly handle missing data. We will use this model to estimate the unadjusted and multivariable-adjusted associations between each of the exposure variables (specified above) and PA level (counts/15s). Covariates that will be included in multivariable models will be pre-determined using a hypothesized directed acyclic graph (DAG).

Objective #3: *Estimate the associations between haemoglobin concentration (Hb) and preschooler physical activity level.*

To address our third objective, summary statistics (including mean, standard deviation, median, range, interquartile range) will be generated for hemoglobin (as a continuous variable) to help identify outliers and biologically implausible data.

Mixed linear modeling with a random intercept will then be used to determine the unadjusted and multivariable-adjusted association between infant hemoglobin and physical activity (mean counts/15s). Similar to objective #2, a priori covariates to be included in the multivariable model will be determined through the development of a DAG.

Using the same procedures identified in Objective #2, we will assess model assumptions and fit.

Objective #4: *Describe sleep quantity and quality in the sample of preschool-aged children in urban Bangladesh*

To address our fourth objective, we will create histograms of the distribution of the data to assess the spread and shape of the frequency distribution of the various available measures of sleep quantity and quality. The number of bins within the histogram will be determined using the equation $1 + 3.3\log_{10}(n)$. A boxplot will then be created to assess the spread, as measured by the median; interquartile range (IQR); range of observations; presence of outliers; and provide additional information about the shape of the data. Finally, a Q-Q plot will be created to compare the frequency distribution of the data to a normal distribution. Using the shape and direction of the curvature, we will assess any deviation from normality.

We will then create summary statistics (including mean/standard deviation or median/range/interquartile range) for:

- Time spent in bed (hours)
- Time "to bed", time "out of bed"
- Sleep quantity
    - Time at which "sleep onset"
    - Total Sleep Time (TST) (hours; min)
    - Total activity counts (cp60s)
- Sleep quality
    - Wake After Sleep Onset (WASO) (min)
    - Awakenings (number/sleep period); Avg awakenings (avg number/sleep period)
    - Movement Index (MI)
    - Fragmentation Index (FI)
    - Sleep Fragmentation Index (SFI)
    - Sleep efficiency (%)

We will also compare distributions across groups stratified by:

- Boys and girls
- BMI categories (underweight, normal, overweight and obese)
- Household food security status (food secure, moderately food insecure; severely food insecure)
- Anemic and non-anemic
- Other maternal and infant characteristics

We will then estimate the frequency of children who met Canadian physical activity 24 Hour Movement Guidelines recommendations on any day, and the average number of days on which children met the recommendations for sleep duration (10-13 hours/day)[57]. Bangladeshi recommended guidelines for preschool-aged children will be used if they are available at the

time of statistical analyses. These data will be compared to published results in similar international, preschooler populations.

This will be done using the same data files collected from the participant; however, we will only use data from non-waking hours. We will use the ActiLife software to analyze all nighttime activity data and derive variables pertaining to sleep quantity and quality.

We will also compare objectively measured sleep periods (based on accelerometry) with parent reported bedtimes and waking times. This will be done using Individual Manual Inspection (IMI) by comparing bedtimes and waking times reported in the Accelerometry & GPS Log to the computed activity and sleep graphs created in the ActiLife software.

10.4.2. **Sensitivity analyses**

To account for the participants' 'adjustment time' to wearing the activity belts, we will exclude the first day of wear-time from analyses.

We will be comparing the wear-time location data from parent-report measures to the data from the GPS logger to evaluate whether parent-report alone provides enough information on the participant's location information. We will assess the GPS data for each participant, specifically looking for substantial coordinate (latitude and longitude) changes. Any deviations from the home coordinates will be cross-referenced to the mother's report by comparing the coordinate's corresponding time stamp and the time indicated by the mother. We will also assess any coordinate changes if the parent indicated that the child left the home.

We will run the same model described in objective 2, however we will stratify by preschooler sex.

We will be comparing the mean cp15s of the Trost and colleagues cut-points [55] to the cut-points established by Pate and colleagues [56], Sirard and colleagues [58], and Van Cauwenberge and colleagues [59]. We will conduct an ANOVA to establish if there is a difference among strata listed above.

11.Data Security and Protection of Subject Confidentiality

Data will be entered and accessed using RedCap (Sick Kids version) with an encrypted laptop and managed on a secure server at the iccdr,b in Dhaka. This laptop will be accessed by the student investigator during the study collection period in Dhaka, and then transported back to Toronto when the student returns post-data collection period. If any data are collected after the student investigator returns to Toronto, data will be securely transferred to Toronto via a

secure web portal. All participants will be assigned a unique study ID number, which will be used to ensure that transferred data is de-identified.

All photographs will be organized, stored, and transferred using the participant's ID to ensure subject confidentiality. Individuals will not be captured in the photographs; however, if any faces or identifiable characteristics are captured accidentally, these images will be blacked out accordingly.

Double data entry by different study personnel and in-built range/consistency checks will be used to optimize data quality. Discrepancies between data enterers will be flagged by RedCap and adjudicated by the student investigator using hardcopies of the data collection forms.

12. Consent Process and Documentation

Written consent will be obtained from a primary caregiver (typically the mother) for all study procedures (Consent Form A for study procedures; Consent Form B for home audit and photographs of the built environment inside and outside of the home). During the initial home visit, the consent form will be read to the participant's caregiver. If all terms are agreed to, the caregiver can either sign the consent form or thumb stamp it using their left thumb. No data collection procedures will commence until written, informed consent is collected.

The study physician will assess the parent/caregiver's capacity to provide consent. This will be based on the study physician's ample experience working with this particular study population.

13. Risks

This is a minimal risk study. There is a low risk of infection following the collection of hemoglobin concentration via finger prick; appropriate study procedures, including the proper cleaning of the finger using an alcohol swab will help to minimize this risk.

The only potential risk/discomfort associated with wearing the accelerometer and GPS logger is the minimal discomfort and weight of the activity belt. During the daily phone calls, study personnel will ask the caregiver of the participant if there are any problems or concerns with their child wearing the devices. This ensures that if there are any compliance issues surrounding wearing the devices, study personnel will be aware of this immediately. If a participant feels uncomfortable wearing the accelerometer, the study personnel will inform their supervisor, and make appropriate changes to increase compliance and decrease participant burden (e.g., encouraging the participant to wear the belt over/under clothes, depending on preference;

attaching stickers to the devices to make them more visually appealing to children; providing the caregiver with a pedometer to model in front of the child while they wear the activity belt). If after making appropriate changes, the participant still does not feel comfortable wearing the activity belt, they can choose to opt-out of the remainder of the study and still receive compensation, if a minimum of 4 days of accelerometer data collection has been obtained.

If a participant feels uncomfortable wearing the GPS logger specifically, we will allow for the caregiver (or whoever is supervising the child) to carry the device on their person, so long as they are with the child during waking hours. In addition, if the caregiver does not feel comfortable disclosing specific locations that their child visits, they can opt to remove the GPS logger from the pouch attached the activity belt for that specific time period. The device will then be returned to the pouch once the participant has left that specific location.

Research ethics approval will be obtained from both the Research Ethics Board at the Hospital for Sick Children in Toronto and the Research Review Committee and Ethical Review Committee at icddr,b in Dhaka.

14. Benefits

Although there are no major health benefits for participating in the study, there is an opportunity to receive medical treatment to individuals who are below the hemoglobin concentration cut-off for anemia.

Additionally, all participants will receive a graphics-based, child-friendly activity report of their data collected from wearing the accelerometers (e.g., average number of daily steps taken, average time spent being active, average time spent being sedentary, etc.). All activity reports will congratulate the participants on their achievements throughout the week and thank them for participating in the study. The activity reports will be hand delivered to the participants after the accelerometry data has been analyzed. This will also be explained to caregivers to provide them with additional information on increasing or maintaining their child's physical activity levels to promote optimal health.

Financial compensation will be provided to caregivers for their time and participation in the study, with a minimum of 4 days of activity data collection.

15. Safety and Adverse Events

Adverse events are not predicted to occur in this study.

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17. Appendix

Table 1 - List of exposure variables and their derivation

Variable	Unit/Categories; Type of variable	Derivation
Total available floor space inside the home	m^2; continuous	Data required (Form 3: Q9b-c): • Averages of the 2 length measures from each room/space inside the home (m) • Averages of the 2 width measure from each room/space inside the home (m) • Averages of the 2 length measures from each large piece of furniture (>1m^2) from inside the home (m) • Averages of the 2 width measure from each large piece of furniture (>1m^2) from inside the home (m) Area of each room/space within the home will be calculated by multiplying the averages of the 2 length by width measures for each room/space in the home (m^2). Area of each large piece of furniture within the home will be calculated by multiplying the averages of the 2 length by width measures for each large piece of furniture in the home (m^2). Areas from any beds will also be calculated and identified. The total area of available space indoors (m^2) will be calculated by summing all of the areas of living space dimensions within the home. The total area of large furniture (m^2) will be calculated by summing all of the areas of furniture obstructions within the home, excluding the areas from any beds. Finally, the area of total available floor space inside of the home (m^2) will be calculated by subtracting the total area of large furniture (m^2) from the total area of available space indoors (m^2). Areas of the beds will be included as available indoor floor space, as they are often used as a play area for children in this setting.
Total available floor space inside and	m^2; continuous	Data required (Form 3: Q11b-c): • Area of the total available floor space inside the home (as described above);

outside of the home		• Averages of the 2 length measures from each enclosed space outside the home (m) • Averages of the 2 width measure from each enclosed space outside the home (m) • Averages of the 2 length measures from each large obstruction (>1m^2) within the enclosed space outside of the home (m) • Averages of the 2 width measures from each large obstruction (>1m^2) within the enclosed space outside of the home (m) Area of the enclosed outdoor space will be calculated by multiplying the averages of the 2 length by width measures for each outdoor space (m^2). Area of each large obstruction outdoors will be calculated by multiplying the averages of the 2 length by width measures for each large obstruction within the enclosed outdoor space (m^2). The total area of enclosed outdoor space (m^2) will be calculated by summing all of the averages of enclosed outdoor space. The total area of large obstructions (m^2) will be calculated by summing all of the areas of large obstructions within the enclosed outdoor space outside of the home. Finally, the area of total available floor space outside of the home (m^2) will be calculated by subtracting the total area of large outdoor obstructions (m^2) from the total area of enclosed outdoor space (m^2). Finally, the summation of the total available floor space inside of the home (m^2) and the total available floor space outside of the home (m^2) will provide the total available floor space inside and outside of the home (m^2).

Total number of physical hazards in or around the home	Count (all integers between 0 and 5); discrete	Data required:
		• Presence of unsafe stairwell (No = 0; Yes = 1)
		  ○ Form 3: Questions 12a, 12b, 12c, and 12d
		  ○ "No" (will receive a score of 0) if:
		  ▪ Q12a = 1

		▪ Q12a = 2; Q12b = 2; Q12c >0.9 m; Q12d = 1 ▪ Q12a = 2; Q12b = 2; Q12c >0.9 m; Q12d = 2 ○ "Yes" (will receive a score of 1) if: ▪ Q12a = 2; Q12c <0.9 m; Q12d = 1 ▪ Q12a = 2; Q12c <0.9 m; Q12d = 2 ▪ Q12a = 2; Q12c >0.9 m; Q12d = 1 • Presence of stove that is accessible to child (No = 0; Yes = 1) ○ Form 3: Questions 13a and 13b ○ "No" (will receive a score of 0) if: ▪ Q13a = 1 ▪ Q13a = 2; Q13b >0.9 m ○ "Yes" (will receive a score of 1) if: ▪ Q13a = 2; Q13b <0.9 m • Presence of unfinished construction (No = 0; Yes = 1) ○ Form 3: Question 14 ○ "No" (will receive a score of 0) if: ▪ Q14 = 1 ○ "Yes" (will receive a score of 1) if: ▪ Q14 = 2 ▪ Q14 = 3 • Presence of uncovered sewage drain, accessible to the child (No = 0; Yes = 1) ○ Form 3: Question 15 ○ "No" (will receive a score of 0) if: ▪ Q15 = 1 ○ "Yes" (will receive a score of 1) if: ▪ Q15 = 2 • Presence of boti in home and storage of item is accessible to the child (No = 0; Yes = 1) ○ Form 3: Questions 16a and 16b ○ "No" (will receive a score of 0): ▪ Q16a = 1 ▪ Q16a = 2; Q16b = 2 ○ "Yes" (will receive a score of 1):

		▪ Q16a = 2; Q16b = 1 Each score from each of the 5 categories will be added to calculate the combined total of hazards present in the home.
Total number of stationary gross-motor activity items	Count (all integers between 0 and 10); discrete	Data required: • Form 3: Questions 17b-c, 18b-c, 19b-c, 20b-c, 21b-c, 22b-c, 23b-c, 24b-c, 25b-c, 26b-c • The number of incidences where both the 'b' question = 2 AND the 'c' section = 2 (e.g., 17b = 2 AND 17c = 2, this indicates that this item is a *stationary* gross-motor activity item) The number of items that satisfy the above rules will be added together to provide this score. The minimum score is 0 items, with a maximum score of 10 items.
Total number of portable gross-motor activity items	Count (all integers between 0 and 10); discrete	Data required: • Form 3: Questions 17b-c, 18b-c, 19b-c, 20b-c, 21b-c, 22b-c, 23b-c, 24b-c, 25b-c, 26b-c • The number of incidences where both the 'b' question = 1 AND the 'c' section = 2 (e.g., 17b = 1 AND 17c = 2, this indicates that this item is a *portable* gross-motor activity item) The number of items that satisfy the above rules will be added together to provide this score. The minimum score is 0 items, with a maximum score of 10 items.
Hemoglobin concentration	g/L; continuous	Hemoglobin concentration data collecting in Form 4: Q24.

Table 2 - List of additional continuous covariates and their derivation

Variable	Unit	Derivation
Body mass index (BMI) (caregiver)	kg/m^2	Data required (Form 2: Q10-13): • Height (m; average of 2 closest height measures) • Weight (kg; average of 2 closest weight measures) BMI will be calculated using the formula: $BMI = weight/height^2$

Body mass index (BMI) z-score (child participant)	(z-score)	Data required (Form 2: Q1-4): • Height (m; average of 2 closest height measures, value will be adjusted by subtracting 0.7 cm if recumbent length was measured) • Weight (kg; average of 2 closest weight measures) Height and weight will be plotted on the WHO body mass index-for-age (BMI-for-age) chart (sex specific; ages 2 to 5 years) to determine participant BMI z-score.
Waist circumference	cm	Average of the 2 closest waist circumference measures (Form 2: Q5,6,14,15)
Body fat percentage (female caregiver)	%	Data required (Form 2: Q10, 11, 12, 13, 17, 18): • Height (m; average of 2 closest height measures) • Weight (kg; average of 2 closest weight measures) • Resistance (ohms measured at 50 kHz; average of the 2 closest resistance measures) 1) Total body water (TBW; kg) will be calculated using the following equation: $TBW = 4.297 + 0.190*Weight + 0.349*Height^2/Resistance$ 2) To determine fat free mass (FFM; kg), TBW will be divided by a hydration factor of 0.732. 3) Fat mass (FM; kg) will be calculated by subtracting FFM from total weight. 4) Body fat percentage will be calculated using the following formula: Body fat % = (FM/Weight)*100%
Body fat percentage (child participant)	%	Data required (Form 2: Q1, 2, 3, 4, 7, 8): • Height (m; average of 2 closest height measures, value will be adjusted by subtracting 0.7 cm if recumbent length was measured) • Weight (kg; average of 2 closest weight measures) • Resistance (ohms measured at 50 kHz; average of the 2 closest resistance measures) 1) Total body water (TBW; kg) will be calculated using the following equation: $TBW = 0.76 + 0.18*Height^2/Resistance + 0.39*Weight$ 2) To determine fat free mass (FFM; kg), TBW will be divided by a hydration factor of 0.775 (males) or 0.779 (females).

		3) Fat mass (FM; kg) will be calculated by subtracting FFM from total weight. 4) Body fat percentage will be calculated using the following formula: Body fat % = (FM/Weight)*100%
Number of children living in the home	Any integer	Data required: • Form 3: Question 3
Household density	People/ m^2	Data required: • Number of adults living in home (Form 3; Question 2) • Number of children living in home (Form 3; Question 3) • Total available floor space inside of the home (m^2, see above in Table 1) The total number of individuals living in the home will be calculated by summing the number of adults living in the home with the number of children living in the home. The total number of individuals living in the home will then by the total available floor space inside of the home to determine household density.
Number of rooms in home	Any integer	Data required: • Form 3: Question 4
Number of screens available to child	Any integer	Data required: • Form 3: Question 8

Table 3 - List of additional categorical covariates and their derivation

Variable	Categories	Derivation
Sex	Male; Female	Screening Form.
Maternal education	No schooling; some or completed primary education; some or completed secondary education; some or completed tertiary education	Data required: • Form 1: Q3-4
Household wealth	Wealth quintiles	Information on household characteristics and ownership of specific household items will be collected in Form 1. Using principle analysis, participants will be categorized into 5 separate categories. The first principle component will be used to assign each

		individual an asset score, with lower scores reflecting ownership of fewer items (i.e., less relative wealth) and higher scores indicative of greater relative wealth. Using the distribution of asset scores in the study population, quintiles will be formed and participants will be categorized appropriately.
Food security status	Food secure; moderately food insecure; severely food insecure	Data required: • Question 18 (Form 4; 1-item food insecurity screening tool; NutriSTEP™)[61] A score of 4 indicates food secure; a score between 2-3 indicates moderately food insecure; a score of 1 indicates severely food insecure. • Question 19 & 20 (Form 4; 2-item food insecurity screening tool; Hager and colleagues, 2010)[62] A combined score of 6 indicates food secure; a combined score of 4-5 indicates moderately food insecure; a combined score of 1-3 indicates severely food insecure.
More than 1 family living in the home	Yes; No	Data required: • Form 3: Question 6a