BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-018380
Article Type:	Cohort profile
Date Submitted by the Author:	28-Jun-2017
Complete List of Authors:	Lee, Albert ; The Chinese University of Hong Kong, School of Public Health and Primary Care; The Chinese University of Hong Kong, Centre for Health Education and Health Promotion Cheung, Calvin; The Chinese University of Hong Kong, Centre for Health Education and Health Promotion Lo, Kenneth; The Chinese University of Hong Kong, School of Public Health and Primary Care Keung, Vera; The Chinese University of Hong Kong, Centre for Health Education and Health Promotion Mui, Lance; The Chinese University of Hong Kong, School of Public Health and Primary Care Tam, Wilson; National University of Singapore
Primary Subject Heading:	Public health
Secondary Subject Heading:	Nutrition and metabolism
Keywords:	NUTRITION & DIETETICS, Community child health < PAEDIATRICS, PUBLIC HEALTH, EPIDEMIOLOGY

Studying Impact of Nutrition on Growth (SING): A Prospective Cohort Study Protocol for Comparing the Health Outcomes of Children with Different Dietary Quality

Albert Lee[1,2,*] Calvin Ka Man Cheung[1], Kenneth Ka Hei Lo[1,2], Vera Mei Wan Keung[1], Lancelot Wai Ho Mui[2], Wilson Wai San Tam[3]

Corresponding author: Albert Lee, Center for Health Education and Health Promotion, Lek Yuen Health Centre, Shatin, Hong Kong, alee@cuhk.edu.hk, 852-26933708

Affiliation:

1: Center for Health Education and Health Promotion, The Chinese University of Hong Kong, 4th Foor, Lek Yuen Health Centre, Shatin, Hong Kong

2: JC School of Public Health and Primary Care, The Chinese University of Hong Kong, Shatin, Hong Kong

3: Alice Lee Centre for Nursing Studies, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Word Count: 3,564

Abstract

Introduction: Poor dietary intake during pregnancy can have negative repercussions on the mother and fetus. This study therefore aims to explore the prospective association between dietary quality (DQ) of preschoolers and their health status in Hong Kong. Body Mass Index as calculated from body weight and height will be the main outcome variable.

Methods and analysis: This will be a prospective cohort study and is expected to recruit 3,539 young children younger than 4 years old. Their diet will be reported by parents by 3-day food diary and their body weight and height will be measured with standardized instruments once a year. Parental questionnaire will also be administered to acquire information about prenatal development and dietary intake before 2 years old of the children, as well as the lifestyle and family background in baseline. DQ will be measured by Healthy Eating Index. Data will be analyzed using SPSS 24. Linear and logistics regression will be used to examine the association of those predictive factors to the outcomes. General Estimating Equation will be used to examine the longitudinal changes of the outcomes.

Ethics and dissemination: This study has been approved by Joint Chinese University of Hong Kong and New Territory East Cluster Clinical Research Ethics Committee (CREC Ref No: 2013-632) Written informed consent will be obtained from all subjects. The results will be published in due course.

Introduction

Description of the condition

Association between early life nutrition and long term health has been raised for decades.[1-3] Numerous studies have been reported the associations between early life nutrition and various health outcomes including obesity,[3] allergy,[4] bone development,[5] immune system,[6] reproductive system,[7] breast cancer risk,[8] etc. The mechanism behind early life nutrition and adult diseases has also been discussed.[9]

Epidemiological and animal studies have revealed that the risk of metabolic syndrome is significantly increased after exposure to suboptimal nutrition during crucial periods of development.[1] Lim and colleagues further highlighted the importance of potential risk factors to which children are exposed during early life and the global burden of non-communicable diseases.[10] Adair and colleagues suggested interventions to increase birth weight and linear growth during the first two years of life and these interventions are likely to result in substantial gains in height and schooling with more protection from development of adult chronic diseases.[11]

Nevertheless, it was reported that rapid weight gain in the first 2 years of life is related to an increased risk of obesity[12] and insulin resistance in later life.[13] Such phenomenon, referred as catch-up dilemma, has been drawn attention to the potential risks and benefits of faster early growth.[11,14,15] The faster relative weight gain after two years of age has little benefit, and weight gain after mid-childhood can result in adverse effects on risk factors of cardiovascular diseases. [11] Wang et al has highlighted that many current interventions on unhealthy eating and weight control have been on lifestyle modification later on in life neglecting the difficulty of neuro-endocrine programming to return to originally set point, and modulation of peri-natal and early postnatal environment would avoid adverse developmental programming of neuro-endocrine systems leading to obesity later on in life.[16] Compensation consumption of high-energy-density food among preschoolers can result in the suboptimal intake of recommended food groups, as shown by a study in Hong Kong.[17]

The importance of this cohort study

Bhuta has called for cohort studies on elements of child development, education, and employment as outcomes, and as crucial future investments that will enable an improved estimation of the effect on human capital.[2] Recent studies have advanced the understanding of neurobiology application to food addiction and obesity; therefore, multilevel interventions that are beyond simple behavioral approaches are necessary to examine the biochemical effects of complex interventions.[18] A cohort study in the United States revealed that factors in early life are associated with children's body mass index (BMI) at eight years of age,[19] whereas a recent review reported that childhood obesity is a predictor of adult obesity.[3]

Aside from these factors, parents have critical roles in the development of their children during the early years. Arredondo et al. revealed that many studies had reported on the relationship between parenting style and children's consumption of healthy food, as well as the amount of consumption.[20] Parenting style was also associated with being overweight in early childhood, as early as the first grade.[21] Many studies in Hong Kong focused on the effects of parenting style on adolescents,[22-26] but no study has focused on the effect of parenting style on physical health.

Although the use of cohort design has numerous advantages in determining the causal relationship between potential risk or protective factors and various health outcomes, some practical difficulties, such as prerequisite of large sample size and long follow up period, limit the use of such design.[27] To our knowledge, two community cohorts are currently used in Hong Kong. These cohorts are the FAMILY cohort[28] and "Children of 1997" birth cohort.[29] The former cohort focuses on happiness, harmony, and health of families,[30-32] whereas the latter focuses on the effect of the first 18 months of life on the health status of children.[29,33,34]

The birth cohort was established 15 years ago, and the breastfeeding practice, nutritional pattern, and lifestyle status have changed over time.[35,36] A knowledge gap also exists in terms of the influence of nutritional pattern and lifestyle practice on the health and

growth of preschoolers. Therefore, both prospective and retrospective studies on children starting early childhood education should be designed to analyze the influence of psychosocial and economic factors during the first 1,000 days of life on health and nutritional status, as well as *the long-term effect of nutrition* on health, functional status, and well-being, along with human capital development mediated by socio-demographic factors.

Objectives

Since no large study has been conducted on a cohort of children from kindergarten to primary school in Chinese population, the aims of this study are to provide comprehensive and reliable data that can be used to describe and understand the physical and mental health development of children during early schooling, as well as how children's early exposures relate to their later development.

Primary objective

Studying the influence of early life nutritional intake and dietary pattern and diet quality as measured by the Healthy Eating Index (HEI) adapted to Hong Kong dietary guidelines, on future health status, including lung function, functional health status, and emotional health.

Secondary objectives

To study:

i. the status of diet and nutrients intake of children in Hong Kong longitudinally;
ii. the correlation of early nutritional intake and childhood obesity;
iii. the influence of school and home environment (physical and social) on childhood health and well-being, as well as nutritional status and development;
iv. the correlation of suboptimal growth and nutrition status with dietary pattern, parenting skills, health literacy of parents, household environment, and suboptimal breast feeding; association of parenting styles with being overweight and nutritional intake.

Methods and Analysis

Study design

This study is a prospective cohort study.[37] The overall approach of reporting the study is consistent with STROBE statement.[38]

Settings

Recruitments were taken place in local kindergartens and nurseries.

Inclusion criteria

Eligible subjects are students younger than 4 years old in local kindergartens and nurseries at the time of recruitment and their parents.

Sampling

A cluster sampling was used in this study,[39] and each kindergarten or nursery was considered as a cluster. A full list of kindergartens and nurseries in Hong Kong were extracted from the Education Bureau of Hong Kong SAR Government.[40] Kindergartens and nurseries were randomly selected from the list.

Procedure

Principals of the selected clusters were approached from late 2014 to early 2015 for approval in study participation. With their consents, all students and their parents in the respective kindergartens and nurseries were invited to participate. Since the study involved a large number of schools and recruitment was started in the middle of the academic year 2014-2015 i.e. Jan 2015, in order to match up the yearly plan of some schools, most of their recruitment were postponed to 1st term of the academic year 2015-2016 i.e. Sep-Dec 2015 and therefore, the subjects were recruited at varied time throughout the year, with the majority being recruited during Sep-Dec 2015. Having obtained the written consent from the parents of the students, the students would undergo baseline assessment of parameters reflecting different outcomes.

Exposure

Exposure was defined as exclusive breastfeeding for 4 months where the information would be collected from the baseline questionnaire. Other exposures included diet high in processed meats or junk food, inadequate intake of particular nutrients according to corresponding cut-offs, and the overall dietary quality as revealed by the Healthy Eating Index).

Sample size estimation

Given that the prevalence of EBF had risen from 6% in 1997 to 14.8% in 2010,[41] we expected the prevalence for EBF would be around 15%. Thus, the ratio for EBF to non-EBF children would be around 15:85 or 3:17.

Previous study reported that short-term exclusive breastfeeding (EBF), that is, <4 months of exclusive breastfeeding, was associated with obesity in five-year-old children. (OR = 1.44; CI = 1.00 to 2.07; $P = 0.050$).[42] As the obesity problem has become more severe, we would expect a larger difference in the prevalence of obesity between EBF and non-EBF groups at the end of the study period. Assuming the OR=1.9, 5% level of significance with ~80% power, the sample size required to detect the difference would be 420 for EBF group and 2,800 non-EBF group given 3:17 ratio. Therefore, 3,280 participants would be needed at the end of the four-year study period. Given a 2.5% annual dropout rate, the sample size needed at the beginning of the study would be around 3,539. According to the Education Bureau, the total number of pre-nursery students in the 2012–2013 academic years was 54,829,[43] therefore, the suggested sample size could cover approximately 6.5% of the students at that age. With assumption of around 20 to 30 pre-nursery students per kindergarten and nursery, around 140 kindergartens and nurseries would be required.

Outcome Measurements

Measurements included 3 components at baseline, namely questionnaire, food diary and physical measurement. Follow-up questionnaire and physical measurement will be collected annually after the baseline data collection. Details of the components at baseline are described below.

Questionnaire

Students' parents were invited to fill the questionnaire and returned to the researcher. Both Chinese and English versions were available for them. There are 3 sections in the baseline questionnaire: Section A is about the Prenatal Development of the Students; Section B is about Dietary Intake of the students from birth to 2 years old; Section C is

about the Lifestyle and Family Background of the students.

Prenatal Development

There are nine questions about the prenatal development of the children including their deliver mode, weeks of gestation, parental drinking and smoking status, maternal health conditions, and weight gain during pregnancy. The weights of the children at birth were also asked.

Dietary Intake from birth to 2 years old

A total of 24 questions are used to examine the dietary intake of the children from birth to 2 years old. The first 5 questions are about the breastfeeding practice when the children were <1 year old while the next 4 questions are about the formula milk feeding practice up to 18 months old. Question 10 to 14 collecte information about when and which kind of solid food had been consumed by the children since birth. Question 15 to 21 enquire the feeding practice on non-infant formula milk, Chinese herbal or botanical preparation drink and nutritional supplement. Question 22 to 24 assesse the frequency of consumption of commercial baby food and pre-packaged or processed food of the subjects since the introduction of solid food to 3 years old. Commercial baby food are categorized into different food groups, including fruits and vegetables, grains, meats, beverages, and snacks. The frequency of consumption ranged from 'None' to '> 4 times per week'.

Lifestyle and family background

There are 22 questions in this section which can be divided into 2 parts. The first groups includes questions related to the lifestyles and habits of the children including number of siblings, living with who, primary carers, sleep pattern, oral practice, activities involvement and general health status. The second part seeks the family background information of the students including questions about the type of housing, working status, occupation, education level, smoking status, birthday, weight and height of parents, and monthly household income.

Food Diary

A 3-day food diary is used to record the dietary intake of the children and the diary was distributed to the parents along with the questionnaire. The parents are advised to include 2 weekdays and 1 weekend or Sunday for the 3-day period and to write down the food items and portions that their children consumed right after each meal. A standard medium bowl as well as an independent instruction booklet named Children's Food Photo Book with food photos in actual size is provided to aid them in filling the food diary. They are also encouraged to take digital photos of the food as supplementary information for researchers' references. A sample of the food diary and instructional booklet are attached. As for the children's meal during school time, parents are notified the two weekdays when teachers would keep records of food intake of participating students in school and researchers also acquire menu from schools directly as supplementary information for the food diary.

Anthropometric assessment

The height of the student is measured without shoes to the nearest 0.1 cm with a portable stadiometer (SECA® 213) and the weight is measured without shoes, to the nearest 0.1 kg, using a digital calibrated floor scale (Tanita® model HD-662). BMI was calculated in kg/m^2.

Follow-up

Follow-up measurement will still include the 3 components and physical measurement will remain the same. Food diary will also be used to capture the dietary pattern of the children.

Pilot study

To ensure the quality of the cohort study, a pilot study was conducted to test the logistics and reliability of selected instruments in the questionnaire. Three kindergartens, involving parents of 104 children, were invited for the pilot study. [44,45]

The parents were invited again to fill in some of the instruments in two-week time, and

the intra-class correlations were computed to examine the test–retest reliability of the instruments.[46] The test–retest reliability of most questions was higher than 0.7, which were suitable for administration in main study.

A sub-sample of 19 parents filled in the food diary instrument with food photos provided. [44] Numbers of foods reported in the food diary and in the photos were compared. Out of 19 parents, 16 of them achieved a high consistency between food diary and food photos (over 90% food items matched).

Upon completion of the pilot study, the procedure was revised in case of any flaw in the logistics. Modification or revision of the instruments was conducted, and details of the amendments were recorded before the start of the main phase of the cohort study.

Data Collection

Questionnaire and anthropometric assessment

Questionnaires will be verified for completeness by the student helpers with nutrition background, and parents will be contacted by phone for any missing information. An Excel database (Microsoft excel 2010) will be created based on the questionnaire and data were entered to the database according to a pre-defined coding scheme.[47] Height and weight will also be entered to the database and BMI would be computed thereafter. The research assistant will conduct data cleaning regularly by computing the frequency figures for each question to identify problematic values.[47]

Cross-checking the problematic values within the questionnaire will be conducted. The research assistant will also performe random checking of the entered data on a regular basis to ensure the data quality.

Food diary

Food records will be checked by a registered dietitian and trained students with nutrition background for any missing or unclear detail. Data will be coded and entered into the

Food Processor program (version 11.0.137) for analysis.

Data analysis

Frequency and percentage will be computed for items with categorical or ordinal responses, whereas mean and standard deviation will be computed for items with continuous responses. For each instrument, the composite score will be calculated. Bivariate correlation will be computed to examine the correlation between the instruments. Independent T-test or ANOVA will be used to examine the difference of scores by demographic and other variables. Linear and logistics regression will be used to examine the association of those predictive factors to the outcomes. General Estimating Equation will be used to examine the longitudinal changes of the outcomes. The analysis will be conducted using IBM SPSS Statistics 24.

Dietary data will be analyzed using the Food Processor program (version 11.0.137) and and Hong Kong food composition tables. Since different cultural foods are served among Hong Kong population, in case of food items with no available local nutritional information, food composition tables for US, China, Taiwan, Japan, Singapore and Australia will be used. . Food composition tables for Hong Kong are accessible from the Centre for Food Safety, HKSAR (www.cfs.gov.hk/english/nutrient/index.shtml), which contain nutrient data of commonly consumed food items in Hong Kong. As for the dietary data obtained in food diaries, the portion of food reported was initially validated by comparing the reported amount of food on the three days with the food photos received for the corresponding day.

The quality of diet will be assessed by Healthy Eating Index (HEI), one of the most extensively studied index (USDA Center for Nutrition Policy and Promotion, 1995)[46] which evaluates the adequacy of intake of food and some nutrients as well as food variety.[48] The calculated index score will be compared among subjects and the associations with health-related outcomes such as growth and weight status will be explored. The adequacy of dietary intake will be assessed by comparing the nutrient intake of the subjects with the Chinese dietary reference intakes developed by the

Chinese Nutrition Society, as well as the estimated average requirement and the reference nutrient intake from the WHO Food and Agricultural Organization (WHO/FAO). Other overseas dietary recommendations may also be applied if daily requirement of a nutrient is not available from both the Chinese Nutrition Society and WHO/FAO. The daily intake of the five food groups will be computed and compared with local recommended intakes for children ages 2 to 6.

Long term growth status

To assess the growth of our subjects longitudinally, the internationally acceptable definition will be adopted.[49-51] The reference was derived from six large nationally representative cross sectional growth studies including US, Hong Kong and Singapore. Age and sex-specific cut-off points will be used to identify children who are underweight, overweight or obese.

Preliminary results

By the end of baseline study recruitment, 5,273 parents of 2-3 years old children from 180 kindergartens and nurseries randomly selected from the 11 districts in Hong Kong agreed to participate. Among a sub-sample of 692 subjects who had completed the data collection stage, the average age of subjects was 3.4 ± 0.4, with 76% of them being 3 years old, 65% being first born, 2.7% being multiple birth, 43% having 1 sibling, and 28% having maid as care givers, 51% living in private property, 54% having family income over $30000. The ever breastfeeding rate among the sub-sample was 82%, with 30% of them having exclusive breastfeeding for more than 6 months.

The most popular reason for weaning breastfeeding among the ever breastfed mothers was not having enough breastmilk. In regard to introduction of solid food, 40% of the sub-sample introduced one new food every 3-6 days when solid food first introduced, however, over 50% of them tended to delay the introduction of potentially allergic food including cheese / yoghurt, peanuts / nuts and seeds, soy products and crustacean shellfish to after 1 or 1.5 years old. 56% of the sub-sample thought their children had picky eating problems, with not eating vegetables being the most common problem. Only 30% of the sub-sample let their children consume nutrition supplement regularly and fish

liver oil was the most popular one given. Baby food was popular among the sub-sample, with over 80% and 60% of them consumed sometimes to usually (at least once per month) baby biscuits and combined meals during 0-2 years respectively. Processed or preserved meats and also junk snacks were also commonly consumed among the sub-sample, with over 50% of them consumed sometimes to usually (at least once per month) processed or preserved meats, sugary drinks, chocolate or candies and desserts, ice-cream and cake or tart after 2 years old.

The rate of overweight and obesity in the sub-sample was 8.7%, with more girls (12.6%) being overweight than boys (4.4%). As for parents' BMI, 54% of fathers and 28% of mothers in the sub-sample were overweighed or obese.

Ethics and Dissemination

This study has been approved by Joint Chinese University of Hong Kong and New Territory East Cluster Clinical Research Ethics Committee (CREC Ref No: 2013-632)

The results of the SING study will provide the backbone information of the diet and nutrients intake of children in Hong Kong. With the information, we can compare the energy intake of the children to the recommendation by the WHO/FAO. In fact, a cross-sectional study was conducted in 2010 and 1,272 children aged from half to 4 years old were recruited .[52] Their results showed that 2.7% children were overweight or obese and the mean energy intake of the children was found to be close to or above the WHO EAR for energy, and the protein intake was adequate. However, due to the cross-sectional design, their study would not be able to examine the longitudinal changes and the casual effect relationship between early nutritional intake and childhood obesity. Therefore, the SING study will be crucial to fill the research gap. In addition, we will develop a healthy eating index that is applicable to the local population. The SING study will also provide insight among the correlation among suboptimal growth and nutrition status with dietary pattern, parenting skills, health literacy of parents, household environment, and suboptimal breast feeding. It would facilitate the development of a research model to study nutrition and health including more distal determinant factors (Figure 1).

The strengths of the study include the use of cluster sampling and a reasonably large sample size, so the results can be generalized to the Hong Kong population. Most instruments adopted in the study are validated and have been widely used in the literature. One of the limitations of the study is that the breastfeeding practice is self-reported at least two years after the birth of the children, and hence recall bias would be inevitable. Another potential limitation is the lack of serum biomarkers to verify the dietary data. The data quality of food diary may vary with the education level of parents. Despite these disadvantages, our study will provide a comprehensive view on the food consumption of Hong Kong children and their health status at the present period.

Contributors

WWT and AL prepared the first draft of manuscript. AL, WWT, CKC, VMK and LWM designed the protocol including selection or refinement of measuring tools. CKC and KKL were involved in pilot testing and are involved in ongoing data collection and analysis. AL is the principal investigator of the project overseeing the conduct of the study. WWT is second principal investigator overseeing the data management. CKC is the coordinator of field work.

Funding Statement

The study is supported by Wyeth Nutrition as research grant to University in accordance to University regulation and guidelines on research. The funding bodies are not involved in data collection and data analysis.

Competing Interests Statement Non declared

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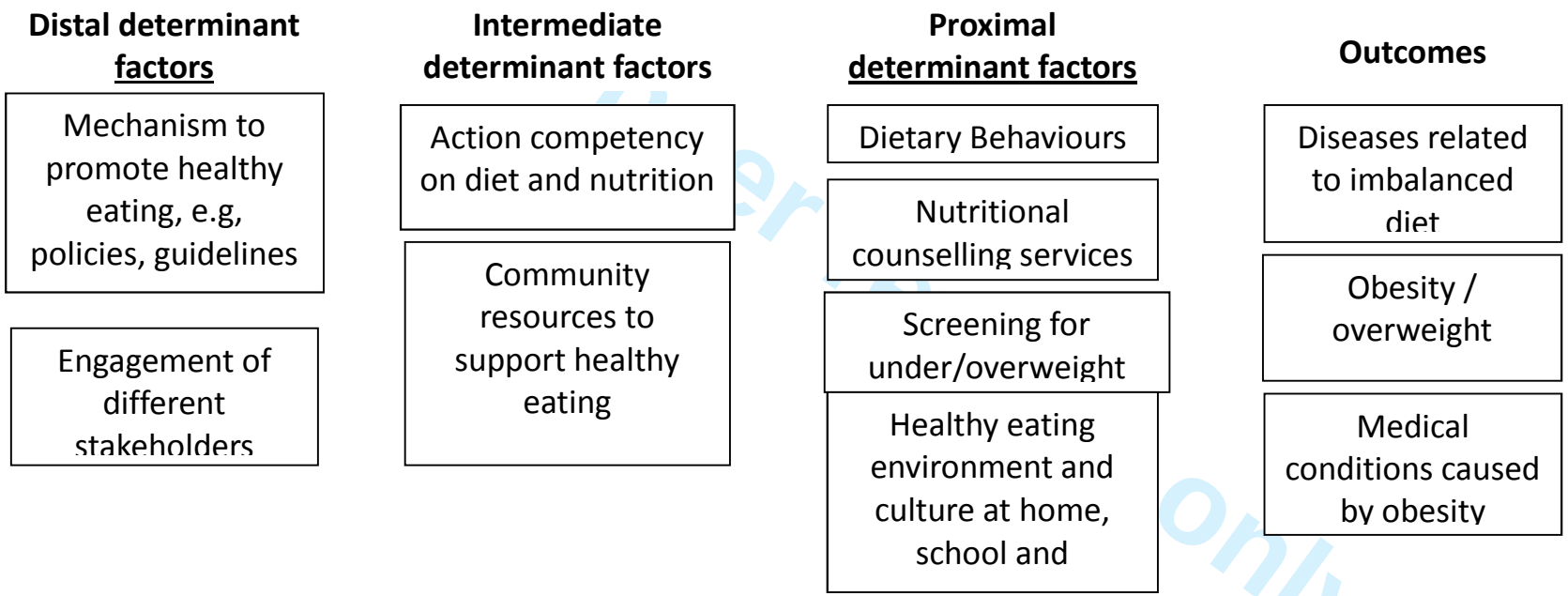
Figure 1. Research model to study nutrition and health



STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract **Page 1 and Page 2**	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale **Page 2-4**	2	Explain the scientific background and rationale for the investigation being reported
Objectives **Page 5**	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design **Page 6**	4	Present key elements of study design early in the paper
Setting **Page 6**	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants **Page 6**	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables **Pages 7 to 9**	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement **Pages 10 to 11**	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias Pages **9 to 10**	9	Describe any efforts to address potential sources of bias
Study size **Page 7**	10	Explain how the study size was arrived at
Quantitative variables **Pages11 to 12**	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods **Pages11 to 12**	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Results		
Participants **Pages 12**	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data **Pages 12-13**	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data **N/A protocol paper**	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results **N/A protocol paper**	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses **N/A protocol paper**	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results **Page 13**	18	Summarise key results with reference to study objectives
Limitations **Page 14**	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation **Pages 13 to 14**	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability **Page 13-14**	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding **Page 15**	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Cohort Profile: Studying Impact of Nutrition on Growth (SING), a Prospective Cohort for Comparing the Health Outcomes of Children with Different Dietary Quality

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-018380.R1
Article Type:	Cohort profile
Date Submitted by the Author:	21-Aug-2017
Complete List of Authors:	Lee, Albert ; The Chinese University of Hong Kong, School of Public Health and Primary Care; The Chinese University of Hong Kong, Centre for Health Education and Health Promotion Cheung, Calvin; The Chinese University of Hong Kong, Centre for Health Education and Health Promotion Lo, Kenneth; The Chinese University of Hong Kong, School of Public Health and Primary Care Keung, Vera; The Chinese University of Hong Kong, Centre for Health Education and Health Promotion Mui, Lance; The Chinese University of Hong Kong, School of Public Health and Primary Care Tam, Wilson; National University of Singapore, Alice Lee Centre for Nursing Studies, Yong Loo Lin School of Medicine
Primary Subject Heading:	Public health
Secondary Subject Heading:	Nutrition and metabolism, Paediatrics, Public health
Keywords:	NUTRITION & DIETETICS, Community child health < PAEDIATRICS, PUBLIC HEALTH, EPIDEMIOLOGY

Cohort Profile: Studying Impact of Nutrition on Growth (SING), a Prospective Cohort for Comparing the Health Outcomes of Children with Different Dietary Quality

Albert Lee[1,2,*] Calvin Ka-man Cheung[1], Kenneth Lo[1,2], Vera Mei-wan Keung[1], Lancelot Wai-ho Mui[2], Wilson WS Tam[3]

Corresponding author: Albert Lee, Center for Health Education and Health Promotion, Lek Yuen Health Centre, Shatin, Hong Kong, alee@cuhk.edu.hk, 852-26933708

Affiliation:

1: Center for Health Education and Health Promotion, The Chinese University of Hong Kong, 4th Foor, Lek Yuen Health Centre, Shatin, Hong Kong

2: JC School of Public Health and Primary Care, The Chinese University of Hong Kong, Shatin, Hong Kong

3: Alice Lee Centre for Nursing Studies, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Word Count: 3,896

Abstract

Introduction: This study aims to explore the prospective association between the dietary quality (DQ) of preschoolers and their health status in Hong Kong, with the Body Mass Index as the main outcome variable.

Methods and analysis: This prospective cohort study has recruited 3,539 children aged between 2 and 4 years old, with a follow-up period of 4 years. Their diet was reported by their parents by a 3-day food diary and their body weight and height were measured yearly with standardized instruments. Questionnaires were administered to parents to acquire information of the children's prenatal development and dietary intake before their age of 2 and of their baseline lifestyle and family backgrounds. The DQ was measured by the Healthy Eating Index as a continuous scale, while the exposure was defined as having a higher dietary quality score. Data was analyzed using SPSS version 24. Linear and logistic regressions were used to examine the association of those predictive factors to the outcomes. Generalized estimating equations will be used to examine the longitudinal changes of the outcomes. A pilot study has been conducted, the preliminary results from which are presented in this cohort profile.

Ethics and dissemination: This study has been approved by the Joint Chinese University of Hong Kong and New Territory East Cluster Clinical Research Ethics Committee (CREC Ref No: 2013-632). Written informed consent was obtained from all subjects. The results will be published in due course.

Strengths and Limitations

- Random sampling with reasonably large size ensuring generalizability
- Validated tools minimizing measurement errors
- Self-reporting tools suffering from recall and social desirable bias

Introduction

Description of the condition

Association between early-life nutrition and long-term health has been raised for decades.[1-3] Numerous studies have been reported the associations between early-life nutrition and normal growth and development (bone development,[4] immune system,[5] reproductive system[6]), acute disease (allergy[7]) and chronic diseases (obesity[8] and breast cancer[9]), etc. For instance, several clinical trials have demonstrated that the intake of calcium and dairy products augments the bone mineral density in children,[10] while the intake of vitamin D positively impacts the development of muscular strength.[11]

Epidemiological and animal studies have revealed that the risk of metabolic syndrome is elevated after exposure to suboptimal nutrition during crucial periods of development.[1] Lim *et. al* further highlighted the significance of potential risk factors to which children are exposed during early life and the global burden of non-communicable diseases.[12] Adair *et. al* suggested interventions to increase birth weight and linear growth during the first two years of life; such interventions are likely to result in substantial gains in height and schooling, with more protection from development of adult chronic diseases.[13]

Nevertheless, it has been reported that rapid weight gain in the first two years of life is related to an increased risk of obesity[14] and insulin resistance in later life.[15] Such a phenomenon, referred to as the catch-up dilemma, has drawn attention to the potential risks and benefits of faster early growth.[13, 16, 17] The faster weight gain after two years of age has little benefit, and weight gain after mid-childhood can result in adverse effects in terms of risk factors of cardiovascular diseases.[13] In a review on early life origins of chronic non-communicable diseases, Wang *et. al.* have highlighted two findings.[18] Firstly, many current interventions on unhealthy eating and weight control have been on lifestyle modification later on in life, thus neglecting the difficulty of neuro-endocrine programming to return to the original set point. Secondly, the modulation of peri-natal and early post-natal environments would avoid adverse developmental programming of the neuro-endocrine system that leads to obesity later on in life.

Compensation consumption of high-energy-density food among preschoolers can result in the suboptimal intake of recommended food groups, as shown by a study in Hong Kong.[19]

The importance of this cohort study

Bhuta has called for cohort studies on elements of child development, education, and employment as outcomes, and as crucial future investments that will enable an improved estimation of the effect on human capital.[2] Recent studies have advanced the understanding of the application of neurobiology to food addiction and obesity; therefore, multilevel interventions beyond simple behavioral approaches are necessary in the examination of the biochemical effects of complex interventions.[20] A cohort study in the United States revealed that factors in early life are associated with children's body mass index (BMI) at eight years of age;[21] whereas a recent review reported that childhood obesity is a predictor of adult obesity.[3]

Aside from these factors, parents have critical roles in their children's development during the early years. Arredondo et al. revealed that many studies had reported the relationship between parental control styles and children's consumption of healthy food, as well as the amount of consumption.[22] An authoritarian parenting style was also associated with being overweight in early childhood, as early as the first grade.[23] Many studies in Hong Kong focused on the effects of the parenting style on adolescents,[24-28] but no study has elucidated the effects of the parenting style on physical health.

Although the use of the cohort design has numerous advantages in providing strong evidence on the causal relationship between potential risks or protective factors and various health outcomes, some practical difficulties, such as the prerequisite of large sample sizes and long follow-up periods, limit its use.[29] To our knowledge, two community cohorts are currently used in Hong Kong: the FAMILY cohort[30] and "Children of 1997" birth cohort.[31] The former focuses on happiness, harmony, and health of families,[32-34] whereas the latter focuses on the effect of the first 18 months of life on the children's health status.[31, 35, 36]

The birth cohort was established in 1997, and the breastfeeding practices, nutritional patterns, and lifestyle status have changed over time.[37, 38] A knowledge gap also exists in terms of the impact of the nutritional patterns and lifestyle practice on the health and growth of preschoolers. Therefore, both prospective and retrospective studies on children starting early childhood education should be designed to analyze the following: the impacts of psychosocial and economic factors during the first 1,000 days of life on health and nutritional status; the long-term effect of nutrition on health, functional status, and well-being; and human capital development mediated by socio-demographic factors.

Objectives

Given the lack of large studies on a cohort of children from kindergarten to primary school in a Chinese population, this study aims to provide comprehensive and reliable data that can be used to describe and understand not only the physical and mental health development of children during early schooling but also how their early exposures relate to their later development.

Primary objective

To investigate the effects of exclusive breastfeeding in infancy on obesity rates among children at the age of 5.

Secondary objectives

To determine:

i. the dietary and nutritional intakes of preschool-aged children longitudinally;
ii. the correlation between early nutritional intake and childhood obesity;
iii. the influence of school and home environment (physical and social) on childhood health and well-being, as well as nutritional status and development;
iv. the correlation between suboptimal growth and nutrition status and factors such as dietary pattern, parenting skills, health literacy of parents, household environment, and suboptimal breast feeding;
v. the association of parenting styles with being overweight and nutritional intake.

Cohort description

Study design

This study is a prospective cohort study.[39] The overall approach of reporting the study is consistent with the STROBE statement.[40]

Settings

Recruitments were conducted in local kindergartens and nurseries, i.e. schools that provide normal education to children aged 2 to 5 in Hong Kong.

Inclusion criteria

Eligible subjects were children aged 2 to 4 in local kindergartens and nurseries at the time of recruitment and their parents.

Sampling

Cluster sampling, in which each kindergarten or nursery was considered as a cluster, was used in this study.[41]. A full list of kindergartens and nurseries in Hong Kong, extracted from the Education Bureau of the Hong Kong SAR Government,[42] was stratified into the 18 districts in Hong Kong. A random number was generated using the "randbetween" function in MS Excel 2010 for each kindergarten and nursery, and then schools were selected according to the magnitude of the assigned random numbers. The number of schools recruited in each district was determined by the sample size estimation as outlined below.

Procedure

The principals of the selected clusters were contacted from late 2014 to early 2015 for approval in the study participation. With their consents, all parents with their children studying in the respective kindergartens and nurseries were invited to participate. Since the study involved numerous schools and recruitment was started in the middle of the academic year 2014–2015, i.e. Jan 2015, in order to match the yearly plan of some schools, most of their recruitment were postponed until the first term of the academic year 2015–2016, i.e. Sep–Dec 2015; therefore, the subjects were recruited at different times throughout the year, with the majority being recruited during Sep-Dec 2015. After the written consent from the parents was obtained, their children underwent baseline

assessment of parameters reflecting different outcomes.

Exposure

Exposure was defined as exclusive breastfeeding (EBF) for four months or longer, this information was collected from the baseline questionnaire. Other exposures included inadequate intake or excessive consumption of particular nutrients (energy intake, protein, total fat, carbohydrates, sodium and calcium) according to the respective cut-offs,[43, 44] and the overall dietary quality derived from the Healthy Eating Index.[45]

Sample size estimation

Given that the prevalence of EBF has risen from 6% in 1997 to 14.8% in 2010,[46] we expected that the prevalence for EBF would be approximately 15%. Thus, the ratio of EBF children to non-EBF ones would be approximately 15:85 or 3:17.

It has been reported that short-term EBF (fewer than 4 months) was associated with obesity in five-year-olds children (OR = 1.44; CI = 1.00 to 2.07; $P = 0.050$).[47] As the obesity problem has become more severe, we would expect a larger difference in the prevalence of obesity between the EBF group and the non-EBF at the end of the study period. Assuming the OR = 1.9 and a level of significance of 5% with ~80% power, the sample size required to detect the difference would be 420 for the EBF group and 2,800 for the non-EBF group, given the 3:17 ratio. Therefore, 3,280 participants would be needed at the end of the four-year study period. Given a 2.5% annual dropout rate, the sample size needed at the beginning of the study would be around 3,539. According to the Education Bureau, the total number of pre-nursery (i.e. schools available for children aged 2 to 3) children in the 2012–2013 academic years was 54,829.[48] Therefore, the suggested sample size could cover approximately 6.5% of the children at that age. With an assumption that there are 20 to 30 pre-nursery children per kindergarten and nursery, approximately 140 kindergartens and nurseries would be required.

Outcome Measurements

Measurements included three baseline components: questionnaires, food diaries and physical measurements. Follow-up questionnaires and physical measurements would be collected annually after the baseline data collection. Details of the baseline components

are described below.

Questionnaire

The children's parents were invited to fill the questionnaires and, upon completion, returned them to the researcher. Both Chinese and English versions were available. There were three sections in the questionnaire on baseline components: Section A concerns the children's prenatal development; Section B concerns their dietary intake from birth to 2 years old; and Section C concerns their lifestyles and family backgrounds.

Prenatal Development

There were nine questions on the children's prenatal development including their delivery mode, weeks of gestation, parental drinking and smoking status, maternal health conditions, and mother's weight gain during pregnancy. The weights of the children at birth were also asked.

Dietary Intake from Birth to 2 years old

A total of 24 questions were used to examine the children's dietary intake from birth to 2 years old. The first five questions revolved around the breastfeeding practice when the children were younger than 1 year old whereas the next four revolved around the formula milk feeding practice up to 18 months old. Questions 10 to 14 collected information of when and which kind of solid food had been consumed by the children since birth. Questions 15 to 21 enquired about the feeding practice on non-infant formula milk, Chinese herbal or botanical preparation drinks and nutritional supplements. Questions 22 to 24 assessed the frequency of consumption of commercial baby food (commercially-prepared pureed food for babies, baby biscuits and desserts) and pre-packaged or processed food of the subjects since the introduction of solid food to the 3-year-olds. Commercial baby food were categorized into different groups, including fruits and vegetables, grains, meats, beverages, and snacks. The frequency of consumption ranged from 'None' to '> 4 times per week'.

Lifestyle and family background

The 22 questions in this section were divided into two parts. The first part included questions related to the children's lifestyles and habits, including the number of siblings, whom they live with, primary carers, sleep patterns, dental practice, activities and general health status. Most questions were in the multiple-choice format, except that the sleep duration was calculated by the difference in reported bedtime and wake time. Items assessing dental practice included whether the children brushed their teeth on their own or needed assistance from parents, and the frequency of teeth-brushing per day. Questions on activities were open-ended for parents to report the types, duration and frequency of extra-curricular activities or hobby classes in which the children were involved. Their general health status was assessed by a single-item question on the times that children required consultation of a doctor. The second part of the section enquired about the family background of the children including the type of housing, and information concerning their parents such as the working status, occupation, education level, smoking status, birthday, weight, height, and monthly household income.

Food Diary

A three-day food diary for recording the children's dietary intake was distributed to the parents, alongside the questionnaire. The parents were advised to include two weekdays and one weekend for the three-day period and to write down the food items and portions that their children consumed right after each meal. A standard medium bowl and an independent instruction booklet named the Children's Food Photo Book with photographs of food in actual sizes were provided to aid them in filling the food diary. They were also encouraged to take digital photos of the food as supplementary information for the researchers' references. Samples of the food diary and instructional booklet are attached. As for the children's meals during school time, parents were notified of the two weekdays when teachers would keep records of food intake of the participating children in school. The researchers also acquired the menus from schools directly as supplementary information for the food diary.

Anthropometric assessment

The height of the children was measured without shoes to the nearest 0.1 cm with a

portable stadiometer (SECA® 213) and the weight was measured without shoes, to the nearest 0.1 kg, using a digital calibrated floor scale (Tanita® model HD-662). BMI was calculated in kg/m^2. Anthropometric measurements were conducted by trained researchers.

Follow-up

Follow-up measurements would be conducted once a year for four years, which would still include the questionnaire for the parents, food diary recording and anthropometric assessment.

Pilot study

To ensure the quality of the cohort study, a pilot study had been conducted to test the logistics and reliability of the selected instruments in the questionnaire. Three kindergartens, involving parents of 104 children, were invited for the pilot study. [49,50]

The parents were invited again to fill in some of the instruments in two weeks' time, and the intra-class correlations were computed to examine the test–retest reliability of the instruments.[51] The test–retest reliability of most questions was higher than 0.7, which were suitable for administration in the main study.

A sub-sample of 19 parents filled in the food diary instrument with food photos provided. [49] The numbers of foods reported in the food diary and in the photos were compared. Out of the 19 parents, 16 of them achieved a high consistency between food diary and food photos (over 90% of the food items matched).

Upon completion of the pilot study, the procedure was revised in case of any logistical flaws. Modification or revision of the instruments was conducted, the details of which were recorded before the start of the main phase of the cohort study.

Data Collection

Questionnaire and anthropometric assessment

The questionnaire was verified for completeness by the student helpers with nutrition backgrounds, and parents were contacted by phone for any missing information. An Excel database (Microsoft excel 2010) was created based on the questionnaire, into which data were entered according to a pre-defined coding scheme.[52] Height and weight were entered to the database and BMI were computed thereafter. The research assistant would conduct data cleaning regularly by computing the frequency figures for each question to identify problematic values.[52]

Cross-checking the problematic values within the questionnaire would be conducted. The research assistant would also periodically perform random checking of the entered data to ensure high data quality.

Food diary

Food diary records would be checked by a registered dietitian and trained students with nutrition background for any missing or unclear details. Data would be coded and entered into the Food Processor program (version 11.0.137) for analysis.

Data analysis

The frequency and percentage would be computed for items with categorical or ordinal responses, whereas the mean and standard deviation would be computed for items with continuous responses. For each instrument, the composite score would be calculated. Bivariate correlation would be computed to examine the correlation between the instruments. Independent T-test or ANOVA would be used to examine the difference of scores by demographic and other variables. Linear and logistic regression would be used to examine the association between those predictive factors and the outcomes. Generalized estimating equations will be used to ascertain the longitudinal changes of the outcomes. The analysis will be conducted using IBM SPSS Statistics version 24.

Dietary data would be analyzed using the Food Processor program (version 11.0.137) and Hong Kong food composition tables. In case of food items with no local nutritional

information available, food composition tables for the US, China, Taiwan, Japan, Singapore and Australia would be used. Food composition tables for Hong Kong are accessible from the Centre for Food Safety, HKSAR (www.cfs.gov.hk/english/nutrient/index.shtml), which contain nutrient data of commonly consumed food items in Hong Kong. As for the dietary data obtained in food diaries, the portion of food reported was initially validated by comparing the reported amount of food during the three days with the food photos received for the corresponding day.

The quality of diet would be assessed by the Healthy Eating Index (HEI), one of the most extensively studied indices which evaluate the conformance to dietary guidelines and the dietary quality.[45] The dietary guidelines component in the HEI will be adapted to the Hong Kong standards for preschoolers. Steps to calculate the HEI scores will include: (a) identifying the set of foods under consideration (foods consumed by Hong Kong preschoolers); (b) determining the amount of each relevant food group, subgroup, and nutrient in the set of foods; (c) deriving the ratios of the scores; and (d) scoring each component (adequacy of total fruit, whole fruit, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant proteins, and fatty acids; moderation of refined grains, sodium and empty calories) using the corresponding standard. [45]

The calculated index score would be compared among subjects and the associations with health-related outcomes such as growth and weight status would be explored. The adequacy of dietary intake (namely energy, protein, total fat, carbohydrates, sodium and calcium) would be assessed by comparing the nutrient intake of the subjects with the Chinese dietary reference intakes developed by the Chinese Nutrition Society, as well as the estimated average requirement and the reference nutrient intake from the WHO Food and Agricultural Organization (WHO/FAO).[43, 44] Other overseas dietary recommendations may also be applied if the daily requirement of a nutrient is not available from both the Chinese Nutrition Society and WHO/FAO. The daily intake of the five food groups would be computed and compared with local recommended intakes for children aged 2 to 6.

Long-term growth status

To assess the growth of our subjects longitudinally, the internationally acceptable definition was adopted.[53-55] The reference was derived from six large nationally representative cross-sectional growth studies including the US, Hong Kong and Singapore. Age and sex-specific cut-off points would be used to identify children who are underweight, overweight or obese.[53-55] For example, at the age of 2, the cut-off points for overweight are 18.41 for boys and 18.02 for girls, while at the age of 3, the cut-off points are 17.89 for boys and 17.56 for girls.

Findings to Date

By the end of the baseline study recruitment, 5,273 parents of the 2- to 4-year-old children from 180 kindergartens and nurseries randomly selected from the 11 districts in Hong Kong have agreed to participate in this study. A total of 693 subjects have completed and returned the questionnaires at the end of the recruitment. (Table 1) The average age of the subjects was 3.4 ± 0.4 years old; among them, 42.9% had 1 sibling, and 26.3% had a maid as their care givers, and 50.6% lived in private property. A total of 31.2% of parents provided EBF for more than 4 months.

The most popular reason for weaning breastfeeding among the mothers was insufficient breast-milk (42.7%). With regard to the introduction of solid food, 15.3% of the sub-samples introduced one type of new food every 4 to 6 days when solid food was first introduced. Not eating vegetables is the most common picky eating habit (24.5%). Among the sub-samples, the rate of being overweight or obese among boys was 4.5% and that among girls was 5.4%.

Ethics and Dissemination

This study has been approved by the Joint Chinese University of Hong Kong and New Territory East Cluster Clinical Research Ethics Committee (CREC Ref No: 2013-632).

Strengths and Limitations

The results of the SING study will provide the backbone information of the dietary and nutritional intake of children in Hong Kong. With the information, we can compare their energy intake to the recommendation by the WHO/FAO. In a cross-sectional study [52] in 2010 involving 1,272 children aged from half to 4 years old, three findings are noteworthy: 2.7% of the participating children were overweight or obese; that their the mean energy intake was found to be close to or above the WHO EAR for energy; and their protein intake was adequate. However, the shortcoming was that the cross-sectional design does not enable an examination of the longitudinal changes and the casual effect relationship between early-life nutritional intake and childhood obesity. Therefore, the SING study will be crucial to fill the research gap. In addition, we will develop a healthy eating index applicable to the local population. The SING study will also provide insights into the correlation between suboptimal growth and nutrition status and factors such as dietary patterns, parenting skills, health literacy of parents, household environment, and suboptimal breast feeding. It would facilitate the development of a research model to study nutrition and health that incorporates more distal determinant factors (Figure 1).

The strengths of the study include the use of cluster sampling and a reasonably large sample size, so the results can be generalized to the Hong Kong population. In addition, most instruments adopted in the study are validated and have been widely used in the literature. One of the limitations of the study is that the breastfeeding practice was self-reported at least two years after childbirth; hence recall bias would be inevitable. Another potential limitation is the lack of serum biomarkers to verify the dietary data. Moreover, the data quality of food diary entries may vary with the education level of parents. Despite these disadvantages, our study will provide a comprehensive view on the food consumption of Hong Kong children and their health status at the present.

Collaboration

Resources have not been forthcoming for providing open access to our data at the present, but we welcome collaboration from interested groups. In order to obtain information on data sharing, we invite researchers to contact us at alee@cuhk.edu.hk.

Contributors

WWT and AL prepared the first draft of manuscript. AL, WWT, CKC, VMK and LWM designed the protocol including the selection or refinement of measuring tools. CKC and KKL were involved in pilot testing and are involved in ongoing data collection and analysis. AL is the principal investigator of the project overseeing the conduct of the study whereas WWT is second principal investigator overseeing the data management. CKC is the coordinator of field work.

Funding Statement

The study is supported by Wyeth Nutrition in the form of a research grant to the University in accordance to University regulation and guidelines on research. The funding bodies are not involved in data collection and data analysis.

Competing Interest Statement None declared.

Figure Legends Figure 1. Research model to study nutrition and health

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Table 1 Demographic features of the participants at the end of recruitment stage (N=693)

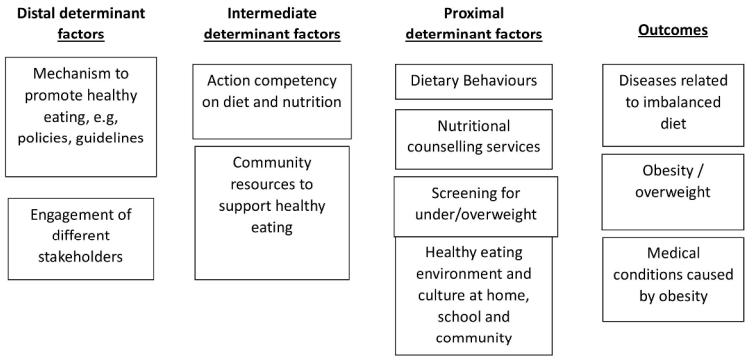
Characteristic	Frequency (%)/Mean±SD [a]
Child Age	3.42±0.43
Age 2	17 (2.5%)
Age 3	332 (47.9%)
Age 4	312 (45.0%)
Children's Gender	
Boys	310 (44.7%)
Girls	296 (42.7%)
Number of siblings	
None	318 (45.9%)
One	297 (42.9%)
Two	62 (8.9%)
Three or more	13 (1.9%)
Main Caregiver(s) (multiple selection)	
Father	209 (30.2%)
Mother	548 (79.1%)
Maid	182 (26.3%)
Housing	
Private Property	351 (50.6%)
Public Estate	146 (21.1%)
Rental property	164 (23.7%)
Others	25 (3.6%)
Breastfeeding practice (multiple selection)	
Breast-fed at early stages, then fed with formula milk	269 (38.8%)
Fed with both breast milk and formula milk	212 (30.6%)
Breast-fed since birth and no formula milk was fed	81 (11.7%)
Fed with formula milk since birth and did no adopt breast-feeding	122 (17.6%)
Duration of exclusive breastfeeding	
Less than 1 week	62 (8.9%)
1-3 weeks	73 (10.5%)
1-2 months	86 (12.4%)
3-4 months	55 (7.9%)
5-6 months	47 (6.8%)

More than 6 months	169 (24.4%)
Reasons for weaning breastfeeding	
It was the time for baby to wean from breastfeeding	97 (14.0%)
Inadequate breast milk secretion by baby's mother	296 (42.7%)
I chose to use formula milk because breast-feeding was more difficult	79 (11.4%)
Baby's mother was occupied by full-time job and not feasible for breast-feeding	151 (21.8%)
Health problem(s) of the mother or baby	25 (3.6%)
Lack of facilities in public areas making breast-feeding inconvenient	33 (4.8%)
Frequency of introducing solid food	
1 new food per week or less often	142 (20.5%)
1 new food every 4-6 days	106 (15.3%)
1 new food every 3 days	116 (16.7%)
1 new food every 2 days	69 (10.0%)
1 new food every day	87 (12.6%)
2 new food every day	28 (4.0%)
3 or more new food every day	6 (0.9%)
Current habit of picky eating (multiple selection)	
Rice	58 (8.4%)
Vegetables	170 (24.5%)
Fruit	37 (5.3%)
Meat	63 (9.1%)
Fish	42 (6.1%)
Egg	40 (5.8%)
Milk	41 (5.9%)
Water	46 (6.6%)
Children being overweight/obese [b]	
Boys	14 (4.5%)
Girls	16 (5.4%)

[a]: The total percentage may not equal to 100% due to missing response.

[b]: The denominator for boys was 310 while that of girls was 296 because not all participants in the subjects have undergone anthropometric assessment.

Figure 1. Research model to study nutrition and health



Research model to study nutrition and health

297x209mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract **Page 1 and Page 2**	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale **Page 2-4**	2	Explain the scientific background and rationale for the investigation being reported
Objectives **Page 5**	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design **Page 6**	4	Present key elements of study design early in the paper
Setting **Page 6**	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants **Page 6**	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables **Pages 7 to 9**	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement **Pages 10 to 11**	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias Pages **9 to 10**	9	Describe any efforts to address potential sources of bias
Study size **Page 7**	10	Explain how the study size was arrived at
Quantitative variables **Pages11 to 12**	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods **Pages11 to 12**	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Results		
Participants **Pages 12**	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data **Pages 12-13**	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data **N/A protocol paper**	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results **N/A protocol paper**	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses **N/A protocol paper**	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results **Page 13**	18	Summarise key results with reference to study objectives
Limitations **Page 14**	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation **Pages 13 to 14**	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability **Page 13-14**	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding **Page 15**	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Cohort Profile: Studying Impact of Nutrition on Growth (SING), a Prospective Cohort for Comparing the Health Outcomes of Young Children with the Dietary Quality Score

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-018380.R2
Article Type:	Cohort profile
Date Submitted by the Author:	11-Oct-2017
Complete List of Authors:	Lee, Albert ; The Chinese University of Hong Kong, School of Public Health and Primary Care; The Chinese University of Hong Kong, Centre for Health Education and Health Promotion Cheung, Calvin; The Chinese University of Hong Kong, Centre for Health Education and Health Promotion Lo, Kenneth; The Chinese University of Hong Kong, School of Public Health and Primary Care Keung, Vera; The Chinese University of Hong Kong, Centre for Health Education and Health Promotion Mui, Lance; The Chinese University of Hong Kong, School of Public Health and Primary Care Tam, Wilson; National University of Singapore, Alice Lee Centre for Nursing Studies, Yong Loo Lin School of Medicine
Primary Subject Heading:	Public health
Secondary Subject Heading:	Nutrition and metabolism, Paediatrics, Public health
Keywords:	NUTRITION & DIETETICS, Community child health < PAEDIATRICS, PUBLIC HEALTH, EPIDEMIOLOGY

Cohort Profile: Studying Impact of Nutrition on Growth (SING), a Prospective Cohort for Comparing the Health Outcomes of Young Children with the Dietary Quality Score

Albert Lee[1,2,*] Calvin Ka-man Cheung[1], Kenneth Lo[1,2], Vera Mei-wan Keung[1], Lancelot Wai-ho Mui[2], Wilson WS Tam[3]

Corresponding author: Albert Lee, Center for Health Education and Health Promotion, Lek Yuen Health Centre, Shatin, Hong Kong, alee@cuhk.edu.hk, 852-26933708

Affiliation:

1: Center for Health Education and Health Promotion, The Chinese University of Hong Kong, 4th Foor, Lek Yuen Health Centre, Shatin, Hong Kong

2: JC School of Public Health and Primary Care, The Chinese University of Hong Kong, Shatin, Hong Kong

3: Alice Lee Centre for Nursing Studies, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Word Count: 3,896

Abstract

Introduction: This study aims to explore the prospective association between the dietary quality (DQ) of preschoolers and their health status in Hong Kong, with the Body Mass Index as the main outcome variable.

Methods and analysis: This prospective cohort study has recruited 3,539 children aged between 2 and 4 years old, with a follow-up period of 4 years. Their diet was reported by their parents by a 3-day food diary and their body weight and height were measured yearly with standardized instruments. Questionnaires were administered to parents to acquire information of the children's prenatal development and dietary intake before their age of 2 and of their baseline lifestyle and family backgrounds. The DQ was measured by the Healthy Eating Index as a continuous scale, while the exposure was defined as having a higher dietary quality score. Data was analyzed using SPSS version 24. Linear and logistic regressions were used to examine the association of those predictive factors to the outcomes. Generalized estimating equations will be used to examine the longitudinal changes of the outcomes. A pilot study has been conducted, the preliminary results from which are presented in this cohort profile.

Ethics and dissemination: This study has been approved by the Joint Chinese University of Hong Kong and New Territory East Cluster Clinical Research Ethics Committee (CREC Ref No: 2013-632). Written informed consent was obtained from all subjects. The results will be published in due course.

Strengths and Limitations

- Random sampling with reasonably large size ensuring generalizability
- Validated tools minimizing measurement errors
- Self-reporting tools suffering from recall and social desirable bias

Introduction

Description of the condition

Association between early-life nutrition and long-term health has been raised for decades.[1-3] Numerous studies have been reported the associations between early-life nutrition and normal growth and development (bone development,[4] immune system,[5] reproductive system[6]), acute disease (allergy[7]) and chronic diseases (obesity[8] and breast cancer[9]), etc. For instance, several clinical trials have demonstrated that the intake of calcium and dairy products augments the bone mineral density in children.[10] Although there were less evidence generated from intervention studies, adequate vitamin D intake could prevent muscle weakness among pediatric population.[11]

Epidemiological and animal studies have revealed that the risk of metabolic syndrome is elevated after exposure to suboptimal nutrition during crucial periods of development.[1] Lim *et. al* further highlighted the significance of potential risk factors to which children are exposed during early life and the global burden of non-communicable diseases.[12] Adair *et. al* suggested interventions to increase birth weight and linear growth during the first two years of life; such interventions are likely to result in substantial gains in height and schooling, with more protection from development of adult chronic diseases.[13]

Nevertheless, it has been reported that rapid weight gain in the first two years of life is related to an increased risk of obesity[14] and insulin resistance in later life.[15] Such a phenomenon, referred to as the catch-up dilemma, has drawn attention to the potential risks and benefits of faster early growth.[13, 16, 17] The faster weight gain after two years of age has little benefit, and weight gain after mid-childhood can result in adverse effects in terms of risk factors of cardiovascular diseases.[13] In a review on early life origins of chronic non-communicable diseases, Wang *et. al.* have highlighted two findings.[18] Firstly, many current interventions on unhealthy eating and weight control have been on lifestyle modification later on in life, thus neglecting the difficulty of neuro-endocrine programming to return to the original set point. Secondly, the modulation of peri-natal and early post-natal environments would avoid adverse developmental programming of the neuro-endocrine system that leads to obesity later on in life.

Compensation consumption of high-energy-density food among preschoolers can result in the suboptimal intake of recommended food groups, as shown by a study in Hong Kong.[19]

The importance of this cohort study

Bhuta has called for cohort studies on elements of child development, education, and employment as outcomes, and as crucial future investments that will enable an improved estimation of the effect on human capital.[2] Recent studies have advanced the understanding of the application of neurobiology to food addiction and obesity; therefore, multilevel interventions beyond simple behavioral approaches are necessary in the examination of the biochemical effects of complex interventions.[20] A cohort study in the United States revealed that factors in early life are associated with children's body mass index (BMI) at eight years of age;[21] whereas a recent review reported that childhood obesity is a predictor of adult obesity.[3]

Aside from these factors, parents have critical roles in their children's development during the early years. Arredondo et al. revealed that many studies had reported the relationship between parental control styles and children's consumption of healthy food, as well as the amount of consumption.[22] An authoritarian parenting style was also associated with being overweight in early childhood, as early as the first grade.[23] Many studies in Hong Kong focused on the effects of the parenting style on adolescents,[24-28] but no study has elucidated the effects of the parenting style on physical health.

Although the use of the cohort design has numerous advantages in providing strong evidence on the causal relationship between potential risks or protective factors and various health outcomes, some practical difficulties, such as the prerequisite of large sample sizes and long follow-up periods, limit its use.[29] To our knowledge, two community cohorts are currently used in Hong Kong: the FAMILY cohort[30] and "Children of 1997" birth cohort.[31] The former focuses on happiness, harmony, and health

of families,[32-34] whereas the latter focuses on the effect of the first 18 months of life on the children's health status.[31, 35, 36]

The birth cohort was established in 1997, and the breastfeeding practices, nutritional patterns, and lifestyle status have changed over time.[37, 38] A knowledge gap also exists in terms of the impact of the nutritional patterns and lifestyle practice on the health and growth of preschoolers. Therefore, both prospective and retrospective studies on children starting early childhood education should be designed to analyze the following: the impacts of psychosocial and economic factors during the first 1,000 days of life on health and nutritional status; the long-term effect of nutrition on health, functional status, and well-being; and human capital development mediated by socio-demographic factors.

Objectives

Given the lack of large studies on a cohort of children from kindergarten to primary school in a Chinese population, this study aims to provide comprehensive and reliable data that can be used to describe and understand not only the physical and mental health development of children during early schooling but also how their early exposures relate to their later development.

Primary objective

To investigate the effects of exclusive breastfeeding in infancy on obesity rates among children at the age of 5.

Secondary objectives

To determine:

i. the dietary and nutritional intakes of preschool-aged children longitudinally;
ii. the correlation between early nutritional intake and childhood obesity;
iii. the influence of school and home environment (physical and social) on childhood health and well-being, as well as nutritional status and development;
iv. the correlation between suboptimal growth and nutrition status and factors such as dietary pattern, parenting skills, health literacy of parents, household environment, and suboptimal breast feeding;
v. the association of parenting styles with being overweight and nutritional intake.

Cohort description

Study design

This study is a prospective cohort study.[39] The overall approach of reporting the study is consistent with the STROBE statement.[40]

Settings

Recruitments were conducted in local kindergartens and nurseries, i.e. schools that provide normal education to children aged 2 to 5 in Hong Kong.

Inclusion criteria

Eligible subjects were children aged 2 to 4 in local kindergartens and nurseries at the time of recruitment and their parents.

Sampling

Cluster sampling, in which each kindergarten or nursery was considered as a cluster, was used in this study.[41]. A full list of kindergartens and nurseries in Hong Kong, extracted from the Education Bureau of the Hong Kong SAR Government,[42] was stratified into the 18 districts in Hong Kong. A random number was generated using the "randbetween" function in MS Excel 2010 for each kindergarten and nursery, and then schools were selected according to the magnitude of the assigned random numbers. The number of schools recruited in each district was determined by the sample size estimation as outlined below.

Procedure

The principals of the selected clusters were contacted from late 2014 to early 2015 for approval in the study participation. With their consents, all parents with their children studying in the respective kindergartens and nurseries were invited to participate. Since the study involved numerous schools and recruitment was started in the middle of the academic year 2014–2015, i.e. Jan 2015, in order to match the yearly plan of some schools, most of their recruitment were postponed until the first term of the academic year 2015–2016, i.e. Sep–Dec 2015; therefore, the subjects were recruited at different times throughout the year, with the majority being recruited during Sep-Dec 2015. After the written consent from the parents was obtained, their children underwent baseline assessment of parameters reflecting different outcomes.

Exposure

Exposure was defined as exclusive breastfeeding (EBF) for four months or longer, this information was collected from the baseline questionnaire. Other exposures included inadequate intake or excessive consumption of particular nutrients (energy intake, protein, total fat, carbohydrates, sodium and calcium) according to the respective cut-offs,[43-45] and the overall dietary quality derived from the Healthy Eating Index.[46]

Sample size estimation

Given that the prevalence of EBF has risen from 6% in 1997 to 14.8% in 2010,[47] we expected that the prevalence for EBF would be approximately 15%. Thus, the ratio of EBF children to non-EBF ones would be approximately 15:85 or 3:17.

It has been reported that short-term EBF (fewer than 4 months) was associated with obesity in five-year-olds children (OR = 1.44; CI = 1.00 to 2.07; $P = 0.050$).[48] As the obesity problem has become more severe, we would expect a larger difference in the prevalence of obesity between the EBF group and the non-EBF at the end of the study period. Assuming the OR = 1.9 and a level of significance of 5% with ~80% power, the sample size required to detect the difference would be 420 for the EBF group and 2,800 for the non-EBF group, given the 3:17 ratio. Therefore, 3,280 participants would be needed at the end of the four-year study period. Given a 2.5% annual dropout rate, the sample size needed at the beginning of the study would be around 3,539. According to the Education Bureau, the total number of pre-nursery (i.e. schools available for children aged 2 to 3) children in the 2012–2013 academic years was 54,829.[49] Therefore, the suggested sample size could cover approximately 6.5% of the children at that age. With an assumption that there are 20 to 30 pre-nursery children per kindergarten and nursery, approximately 140 kindergartens and nurseries would be required.

Outcome Measurements

Measurements included three baseline components: questionnaires, food diaries and physical measurements. Follow-up questionnaires and physical measurements would be collected annually after the baseline data collection. Details of the baseline components are described below.

Questionnaire

The children's parents were invited to fill the questionnaires and, upon completion, returned them to the researcher. Both Chinese and English versions were available. There were three sections in the questionnaire on baseline components: Section A concerns the children's prenatal development; Section B concerns their dietary intake from birth to 2 years old; and Section C concerns their lifestyles and family backgrounds.

Prenatal Development

There were nine questions on the children's prenatal development including their delivery mode, weeks of gestation, parental drinking and smoking status, maternal health conditions, and mother's weight gain during pregnancy. The weights of the children at birth were also asked.

Dietary Intake from Birth to 2 years old

A total of 24 questions were used to examine the children's dietary intake from birth to 2 years old. The first five questions revolved around the breastfeeding practice when the children were younger than 1 year old whereas the next four revolved around the formula milk feeding practice up to 18 months old. Questions 10 to 14 collected information of when and which kind of solid food had been consumed by the children since birth. Questions 15 to 21 enquired about the feeding practice on non-infant formula milk, Chinese herbal or botanical preparation drinks and nutritional supplements. Questions 22 to 24 assessed the frequency of consumption of commercial baby food (commercially-prepared pureed food for babies, baby biscuits and desserts) and pre-packaged or processed food of the subjects since the introduction of solid food to the 3-year-olds. Commercial baby food were categorized into different groups, including fruits and vegetables, grains, meats, beverages, and snacks. The frequency of consumption ranged from 'None' to '> 4 times per week'.

Lifestyle and family background

The 22 questions in this section were divided into two parts. The first part included

questions related to the children's lifestyles and habits, including the number of siblings, whom they live with, primary carers, sleep patterns, dental practice, activities and general health status. Most questions were in the multiple-choice format, except that the sleep duration was calculated by the difference in reported bedtime and wake time. Items assessing dental practice included whether the children brushed their teeth on their own or needed assistance from parents, and the frequency of teeth-brushing per day. Questions on activities were open-ended for parents to report the types, duration and frequency of extra-curricular activities or hobby classes in which the children were involved. Their general health status was assessed by a single-item question on the times that children required consultation of a doctor. The second part of the section enquired about the family background of the children including the type of housing, and information concerning their parents such as the working status, occupation, education level, smoking status, birthday, weight, height, and monthly household income.

Food Diary

A three-day food diary for recording the children's dietary intake was distributed to the parents, alongside the questionnaire. The parents were advised to include two weekdays and one weekend for the three-day period and to write down the food items and portions that their children consumed right after each meal. A standard medium bowl and an independent instruction booklet named the Children's Food Photo Book with photographs of food in actual sizes were provided to aid them in filling the food diary. They were also encouraged to take digital photos of the food as supplementary information for the researchers' references. Samples of the food diary and instructional booklet are attached. As for the children's meals during school time, parents were notified of the two weekdays when teachers would keep records of food intake of the participating children in school. The researchers also acquired the menus from schools directly as supplementary information for the food diary.

Anthropometric assessment

The height of the children was measured without shoes to the nearest 0.1 cm with a portable stadiometer (SECA® 213) and the weight was measured without shoes, to the

nearest 0.1 kg, using a digital calibrated floor scale (Tanita® model HD-662). BMI was calculated in kg/m^2. Anthropometric measurements were conducted by trained researchers.

Follow-up

Follow-up measurements would be conducted once a year for four years, which would still include the questionnaire for the parents, food diary recording and anthropometric assessment. Regular contact with participants would be made to maintain them in the cohort. The demographic features of subjects who have returned the questionnaires in each follow-up would be compared with baseline data to calculate the loss to follow-up and examine potential attrition bias.

Pilot study

To ensure the quality of the cohort study, a pilot study had been conducted to test the logistics and reliability of the selected instruments in the questionnaire. Three kindergartens, involving parents of 104 children, were invited for the pilot study. [50,51]

The parents were invited again to fill in some of the instruments in two weeks' time, and the intra-class correlations were computed to examine the test–retest reliability of the instruments.[52] The test–retest reliability of most questions was higher than 0.7, which were suitable for administration in the main study.

A sub-sample of 19 parents filled in the food diary instrument with food photos provided. [50] The numbers of foods reported in the food diary and in the photos were compared. Out of the 19 parents, 16 of them achieved a high consistency between food diary and food photos (over 90% of the food items matched).

Upon completion of the pilot study, the procedure was revised in case of any logistical flaws. Modification or revision of the instruments was conducted, the details of which were recorded before the start of the main phase of the cohort study.

Data Collection

Questionnaire and anthropometric assessment

The questionnaire was verified for completeness by the student helpers with nutrition backgrounds, and parents were contacted by phone for any missing information. An Excel database (Microsoft excel 2010) was created based on the questionnaire, into which data were entered according to a pre-defined coding scheme.[53] Height and weight were entered to the database and BMI were computed thereafter. The research assistant would conduct data cleaning regularly by computing the frequency figures for each question to identify problematic values.[53]

Cross-checking the problematic values within the questionnaire would be conducted. The research assistant would also periodically perform random checking of the entered data to ensure high data quality.

Food diary

Food diary records would be checked by a registered dietitian and trained students with nutrition background for any missing or unclear details. Data would be coded and entered into the Food Processor program (version 11.0.137) for analysis.

Data analysis

The frequency and percentage would be computed for items with categorical or ordinal responses, whereas the mean and standard deviation would be computed for items with continuous responses. For each instrument, the composite score would be calculated. Bivariate correlation would be computed to examine the correlation between the instruments. Independent T-test or ANOVA would be used to examine the difference of scores by demographic and other variables. Linear and logistic regression would be used to examine the association between those predictive factors and the outcomes. Generalized estimating equations will be used to ascertain the longitudinal changes of the outcomes. The analysis will be conducted using IBM SPSS Statistics version 24.

Dietary data would be analyzed using the Food Processor program (version 11.0.137) and Hong Kong food composition tables. In case of food items with no local nutritional information available, food composition tables for the US, China, Taiwan, Japan, Singapore and Australia would be used. Food composition tables for Hong Kong are accessible from the Centre for Food Safety, HKSAR (www.cfs.gov.hk/english/nutrient/index.shtml), which contain nutrient data of commonly consumed food items in Hong Kong. As for the dietary data obtained in food diaries, the portion of food reported was initially validated by comparing the reported amount of food during the three days with the food photos received for the corresponding day.

The quality of diet would be assessed by the Healthy Eating Index (HEI), one of the most extensively studied indices which evaluate the conformance to dietary guidelines and the dietary quality.[46] The dietary guidelines component in the HEI will be adapted to the Hong Kong standards for preschoolers. Steps to calculate the HEI scores will include: (a) identifying the set of foods under consideration (foods consumed by Hong Kong preschoolers); (b) determining the amount of each relevant food group, subgroup, and nutrient in the set of foods; (c) deriving the ratios of the scores; and (d) scoring each component (adequacy of total fruit, whole fruit, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant proteins, and fatty acids; moderation of refined grains, sodium and empty calories) using the corresponding standard. [46]

The calculated index score would be compared among subjects and the associations with health-related outcomes such as growth and weight status would be explored. The adequacy of dietary intake (namely energy, protein, total fat, carbohydrates, sodium and calcium) would be assessed by comparing the nutrient intake of the subjects with the Chinese dietary reference intakes developed by the Chinese Nutrition Society, as well as the estimated average requirement and the reference nutrient intake from the WHO Food and Agricultural Organization (WHO/FAO).[43, 44] The daily intake of the five food groups would be computed and compared with local recommended intakes for children aged 2 to 6.

Long-term growth status

To assess the growth of our subjects longitudinally, the internationally acceptable definition was adopted.[54-56] The reference was derived from six large nationally representative cross-sectional growth studies including the US, Hong Kong and Singapore. Age and sex-specific cut-off points would be used to identify children who are underweight, overweight or obese.[54-56] For example, at the age of 2, the cut-off points for overweight are 18.41 for boys and 18.02 for girls, while at the age of 3, the cut-off points are 17.89 for boys and 17.56 for girls.

Findings to Date

By the end of the baseline study recruitment (February 2016), 5,273 parents of the 2- to 4-year-old children from 180 kindergartens and nurseries randomly selected from the 11 districts in Hong Kong have agreed to participate in this study by returning the signed consent form. A total of 3,223 subjects have completed and returned the questionnaires. The data entry of the questionnaire was just completed. Table 1 has provided the basic demographic features of the participants. The majority of the participating children were 3 years old (74.5%). Mothers were the most common caregiver of the children (95.1%). A total of 39.0% parents breast-fed their children at early stages, and then fed with formula milk. Vegetables rejection was the most common picky eating habit (26.2%).

Ethics and Dissemination

This study has been approved by the Joint Chinese University of Hong Kong and New Territory East Cluster Clinical Research Ethics Committee (CREC Ref No: 2013-632).

Strengths and Limitations

The results of the SING study will provide the backbone information of the dietary and nutritional intake of children in Hong Kong. With the information, we can compare their energy intake to the recommendation by the WHO/FAO. In a cross-sectional study in 2010 involving 1,272 children aged from half to 4 years old,[57] three findings are noteworthy: 2.7% of the participating children were overweight or obese; that their the

mean energy intake was found to be close to or above the WHO EAR for energy; and their protein intake was adequate. However, the shortcoming was that the cross-sectional design does not enable an examination of the longitudinal changes and the casual effect relationship between early-life nutritional intake and childhood obesity. Therefore, the SING study will be crucial to fill the research gap. In addition, we will develop a healthy eating index applicable to the local population. The SING study will also provide insights into the correlation between suboptimal growth and nutrition status and factors such as dietary patterns, parenting skills, health literacy of parents, household environment, and suboptimal breast feeding. It would facilitate the development of a research model to study nutrition and health that incorporates more distal determinant factors (Figure 1).

The strengths of the study include the use of cluster sampling and a reasonably large sample size, so the results can be generalized to the Hong Kong population. In addition, most instruments adopted in the study are validated and have been widely used in the literature. One of the limitations of the study is that the breastfeeding practice was self-reported at least two years after childbirth; hence recall bias would be inevitable. Another potential limitation is the lack of serum biomarkers to verify the dietary data. Moreover, the data quality of food diary entries may vary with the education level of parents. Despite these disadvantages, our study will provide a comprehensive view on the food consumption of Hong Kong children and their health status at the present.

Collaboration

Resources have not been forthcoming for providing open access to our data at the present, but we welcome collaboration from interested groups. In order to obtain information on data sharing, we invite researchers to contact us at alee@cuhk.edu.hk.

Contributors

WWT and AL prepared the first draft of manuscript. AL, WWT, CKC, VMK and LWM designed the protocol including the selection or refinement of measuring tools. CKC and

KKL were involved in pilot testing and are involved in ongoing data collection and analysis. AL is the principal investigator of the project overseeing the conduct of the study whereas WWT is second principal investigator overseeing the data management. CKC is the coordinator of field work.

Funding Statement

The study is supported by Wyeth Nutrition in the form of a research grant to the University in accordance to University regulation and guidelines on research. The funding bodies are not involved in data collection and data analysis.

Competing Interest Statement None declared.

Figure Legends Figure 1. Research model to study nutrition and health

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Table 1 Demographic features of the participants at the end of recruitment (N=3223)

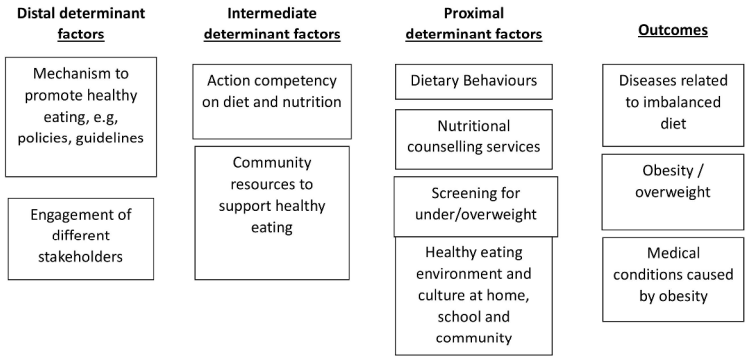
Characteristic	Frequency (%)/Mean±SD [a]
Child Age (in years)[b]	3.38 ± 0.45
Aged 2	588 (18.2%)
Aged 3	2402 (74.5%)
Aged 4	198 (6.1%)
Non-response	35 (1.1%)
Children's Gender [c]	
Male	1669 (51.8%)
Female	1522 (47.2%)
Non-response	32 (1.0%)
Main Caregiver(s) (multiple selection)	
Father	2906 (90.1%)
Mother	3065 (95.1%)
Maid	889 (27.6%)
Breastfeeding practice (multiple selection)	
Breast-fed at early stages, then fed with formula milk	1258 (39.0%)
Fed with both breast milk and formula milk	897 (27.8%)
Breast-fed since birth and no formula milk was fed	432 (13.4%)
Fed with formula milk since birth and did no adopt breast-feeding	617 (19.1%)
Current habit of picky eating (multiple selection)	
Rice	264 (8.2%)
Vegetables	845 (26.2%)
Fruit	205 (6.4%)
Meat	383 (11.9%)
Fish	207 (6.4%)
Egg	228 (7.1%)
Milk	156 (4.8%)
Water	268 (8.3%)

[a]: The total percentage may not equal to 100% due to non-response or multiple selection questions.

[b]: Non-response originated from parents who did not report the birthdate of their children.

[c]: Non-response originated from children who have not undergone anthropometric measurements.

Figure 1. Research model to study nutrition and health



Research model to study nutrition and health

297x209mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2-5
	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants **Page 6**	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*7-9*
Bias Pages	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11-13
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	11
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	10
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking	10

		account of sampling strategy	
		(*e*) Describe any sensitivity analyses	NA
Results			**Page number**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	13
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	19
		(b) Indicate number of participants with missing data for each variable of interest	19
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	13-14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.