**Evaluation of preconception dietary patterns among women enrolled in a multi-site study** 1,2,3

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Footnotes to the title

1 **List of abbreviations**: **BMI**: body mass index, **HbA1c**: glycated haemoglobin A1c, **HDL-cholesterol**: high density lipoprotein cholesterol, **HOMA-2-IR**: updated Homeostasis Model Assessment for insulin resistance (HOMA-2-IR), **hs-CRP**: high-sensitivity C-reactive protein, **LDL-cholesterol**: low density lipoprotein cholesterol, **NiPPeR**: Nutritional Intervention Preconception and During Pregnancy to Maintain Healthy Glucose Metabolism and Offspring Health; **NZ**: New Zealand, **SG**: Singapore, **UK**: United Kingdom

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**Abstract**

**Background**

Diet indices are widely used in nutritional research across communities but do not “capture” the full extent of diet variability across multiple countries. Empirically-derived dietary patterns can provide additional information as they reflect combinations of foods potentially associated with health outcomes. Limited studies have evaluated preconception dietary patterns among heterogeneous populations.

**Objectives**

In the multi-site NiPPeR study, secondary aims included: (1) derive pooled and site-specific preconception dietary patterns, and (2) evaluate these patterns using anthropometric measures and metabolic biomarkers.

**Methods**

Women planning pregnancy (n=1720) in the United Kingdom, Singapore and New Zealand completed interviewer-administered harmonized food-frequency and lifestyle questionnaires at recruitment. Across-cohort (“pooled”) and site-specific dietary patterns were derived, and associations between dietary pattern scores and BMI, waist to hip ratio, plasma lipids and glycemia assessed using multivariable linear regression, expressing results as standard deviation change in outcome per standard deviation change in dietary pattern score.

**Results**

The pooled analysis identified three dietary patterns: ‘Vegetables/Fruits/Nuts’ (‘Healthy’), ‘Fried potatoes/Processed meat/Sweetened beverages’ (‘Less Healthy’) and ‘Fish/Poultry/Noodles/Rice’ (‘Mixed’). The ‘Healthy’ and ‘Less Healthy’ pooled pattern scores were highly correlated with their corresponding site-specific dietary pattern scores (‘Healthy’: ρ=0.87-0.93, ‘Less Healthy’: ρ=0.65-0.88). Women with higher scores for the ‘Healthy’ pooled pattern had a lower waist to hip ratio [Standardized beta (95% CI): -0.10 (-0.18, -0.01)]; those with higher scores for the ‘Less Healthy’ pooled pattern had a higher BMI [0.17 (0.09, 0.24)], higher LDL cholesterol [0.10 (0.01, 0.19)] and less optimal glucose profiles. However, we noted higher adherence to the ‘Healthy’ pooled pattern with higher BMI.

**Conclusions**

The ‘Healthy’ and ‘Less Healthy’ pooled patterns were comparable to the corresponding site-specific patterns. While the associations between these patterns and objective anthropometric/metabolic measures were largely in the expected directions, future studies are required to confirm these findings.

This trial is registered at ClinicalTrials.gov (NCT02509988).

Keywords: preconception, dietary patterns, multi-site, FFQ, evaluation

**Summary**

In a multi-site study, pooled preconception dietary patterns were strongly correlated with site-specific dietary patterns, and were associated with objective anthropometric/metabolic measures.

**Introduction**

Unlike diet quality indices, comparing exploratory (data driven) dietary patterns across multiple countries or ethnic groups is more complex (1). Nevertheless, these exploratory dietary patterns remain informative as they provide insights into existing overall diets that could be associated with health outcomes (2, 3). Several studies involving healthy adults (4), the elderly (5) and pregnant women (6) have adopted harmonization methods to enable generalizable exploratory dietary patterns to be defined across various countries or study populations. However, it remains uncertain whether the harmonized patterns adequately represent the populations in question and whether harmonizing these patterns will result in the loss of site-specific dietary information. This led one study to internally validate the harmonized ‘Plant-based’ exploratory dietary pattern against self-reported vegetarian status and externally validating the harmonized pattern against the modified Alternative Healthy Eating Index (mAHEI) (6). To date, harmonized exploratory dietary patterns has mostly been evaluated using subjective self-reported measures (6) or have yet to be evaluated when they were generated using data from validated country- or site-specific food frequency questionnaires (FFQs) (4, 5).

Biomarkers have been commonly used in validating dietary patterns in single population studies (7). For example, while increasing adherence to the ‘Healthy’/’Prudent’ patterns tended to be associated with more favourable metabolic biomarker profiles (2, 8-12), increasing adherence to the ‘Less Healthy’/’Western’ patterns were associated with less favorable biomarker profiles (8, 11-13). Biomarkers have the benefit of being objective as they are unaffected by self-reporting bias (7). Additionally, they have been shown to reveal ethnic-specific differences in associations between diet and health outcomes, likely due to biological differences in metabolism or the subtle differences in diets across ethnic groups (2, 9). To our knowledge, biomarkers have not previously been used for validating harmonized dietary patterns among women planning pregnancy. Given the emerging evidence on the links between preconception nutrition and subsequent pregnancy/offspring health outcomes, studies examining dietary patterns during the preconception phase is of interest (14, 15).

We leveraged a multi-site preconception study, the NiPPeR (Nutritional Intervention Preconception and During Pregnancy to Maintain Healthy Glucose Metabolism and Offspring Health) study, to harmonize baseline dietary data from women planning pregnancy across three sites. Following this, we evaluated the derived harmonized (pooled) and site-specific dietary patterns using multiple objective measurements including anthropometric measures and metabolic biomarkers. Additionally, we evaluated whether the pooled dietary patterns led to the loss of site-specific dietary information and if these patterns adequately represent the populations in question. These are secondary outcomes of interest in the NiPPeR study.

**Methods**

**Study design and participants**

All information used for this study was collected at recruitment to the NiPPeR study, which is prior to start of any intervention. The NiPPeR study is a double blind randomized trial that compared the effects of a standard nutritional drink with the effects of a nutritional supplement containing standard preconception/pregnancy micronutrients (folic acid, iron, calcium, iodine, β-carotene) with an enriched supplement (additionally containing myo-inositol, vitamin D, riboflavin, vitamin B6, vitamin B12, zinc and the probiotics *Lactobacillus rhamnosus* and *Bifidobacterium animalis sp. Lactis* with additional nutrients). The primary outcome was the maintenance of healthy glucose levels during pregnancy (16).

Participants were healthy women, aged between 18 to 38 years, who were planning pregnancy in Southampton, United Kingdom (UK), Singapore (SG) and Auckland, New Zealand (NZ). Women who (a) were already known to have diabetes (type I or type II), (b) were taking oral steroids or anticonvulsant medication, (c) were seeking assisted fertility treatment (apart from clomiphene and letrozole), (d) were taking hormonal contraceptives and (e) were seeking treatment for Human Immunodeficiency Virus (HIV), Hepatitis B or C in the past month and (f) had known serious food allergies were excluded. Further details of the NiPPeR trial have been published elsewhere (16). Standardized protocols for data and sample collection were applied across sites.

The NiPPeR study has been granted ethical approval by the Research Ethics Committees at each of the three study sites (Southampton: Health Research Authority NRES Committee South Central Research Ethics Committee (REC), reference 15/SC/0142), the Health and Disability Ethics Committee (HDEC) (New Zealand) reference 15/NTA/21 and the National Healthcare Group Domain Specific Review Board (NHG DSRB) (Singapore) reference 2015/ 00205). Participation was on a voluntary (unpaid) basis, and participants provided written informed consent for future use of their data in published research including the analyses described in this paper.

**Dietary assessment**

Validated semi-quantitative food frequency questionnaires (FFQs) for adults from the UK (100 item) (17), SG (92 item) (18) and NZ (83 item) (19) were harmonized for the NiPPeR study. This was done by comparing the items from the three FFQs and aligning them across sites. Upon categorizing the FFQ items into either core or site-specific food groups, there were a total of 41 core food groups that were largely similar across all three sites (**Supplementary Table 1**). FFQ items that were unique to each site or to only two sites were also identified and grouped into site-specific food groups. In total, there were 51, 47 and 53 food groups belonging to the UK, SG and NZ FFQ, respectively. This classification process and the administration approach of the FFQs was discussed and agreed by the investigators from all three sites prior to the dietary assessment. Further details can be found in the **Supplementary Text**.

Across all 3 sites, usual dietary intakes during the month preceding enrollment were assessed using the harmonized semi-quantitative FFQs, which were administered in-person by trained research staff. For each food item, participants were asked to indicate their frequency of consumption in an open-ended format (with options of Never, Frequency per month, Frequency per week or Frequency per day) of standard portions of foods and beverages. Subsequently, responses for all FFQ line items were standardized to daily intakes. Total daily energy intakes were calculated for each participant using site-specific food composition databases to accommodate the distinct aspects of the site-specific food items. The exclusion of dietary misreporting using energy intakes was discussed and agreed by all investigators. The lower limit (500 kcal) was based on existing literature (20), while the upper limit (7000 kcal) was based on energy intakes of obese women enrolled in the HUMBA trial (unpublished findings) (21).

**Sociodemographic and lifestyle factors**

Trained research staff conducted in-person interviews with enrolled participants following recruitment. Information including sociodemographic (e.g. age, annual household income) and lifestyle behaviors (e.g. alcohol consumption, smoking status, physical activity and sedentary behaviors) were collected. Ethnicity was categorized into five groups: White, Chinese, Malay and South Asian and, a fifth ‘Other ethnicity’ group (including Polynesians, Blacks and other Asians). Participants were asked about the number of days they engaged in moderate and vigorous physical activity in the past 7 days. Additionally, participants also reported the number of days and average amount of time spent on sedentary behaviours (sitting time at leisure, viewing television and use of electronic devices in the past 7 days and sitting time at work in an average working day). Total sitting time was derived as the total daily time spent sitting at work and sitting at leisure for the past week. Total screen time was derived as the total daily time spent on viewing television and using electronic devices for the past week.

**Anthropometric and metabolic measures**

During the first preconception visit, weight (Seca 899 scales) and height (Leicester height measure) were measured to the nearest 0.1 kg and 0.1 cm, respectively, for calculation of body mass index (BMI, kg/m2), together with measurements of waist and hip circumferences (cm), used for calculation of waist to hip ratio. Anthropometric measures were taken in triplicates and the mean value for each measure was recorded.

Plasma glucose (fasting, 30-min and 120-min) in a 75g oral glucose load tolerance test and glycated haemoglobin (HbA1c) were measured by a single laboratory at each site, with uniform external quality assurance as per the Royal College of Pathologists of Australasia Quality Assurance Program. Serum concentrations of fasting insulin, high sensitivity C-reactive protein (hsCRP) and fasting plasma lipids (triglycerides, HDL-cholesterol and LDL-cholesterol) were batch analysed in a single laboratory (cobas; Roche Diagnostics). The homeostasis model assessment index for insulin resistance (HOMA2-IR) was calculated using fasting plasma glucose and fasting serum insulin (22).

**Statistical analyses**

*Derivation of pooled and site-specific dietary patterns*

Factor analysis was used to derive the underlying preconception dietary patterns. The Kaiser-Meyer-Olkin Measure of sampling adequacy (KMO test) and Bartlett’s test of sphericity tests were first performed to determine if the data was suitable for factor analysis (23). Varimax rotation was next performed to ensure that the factors derived were independent of one another and to improve factor interpretability (24). The choice of the number of factors to retain was based on the break point of the Scree plot, an eigenvalue of above 1 and factor interpretability. Factor loadings, estimated using the principal factor method, are the correlation coefficients of each food group and the derived dietary pattern; hence, higher factor loadings indicate a greater contribution of a particular food group to that derived pattern. For simplicity, only food groups with factor loadings of at least 0.25 were presented. Subsequently, dietary pattern scores for each participant were calculated by summing the standardized intake of food groups (frequency/day) weighted by their regressed factor loadings, giving each participant a score for each derived pattern. A higher dietary pattern score to a specific dietary pattern indicates greater adherence to that derived pattern. The measures of suitability of data for factor analysis and scree plots of the pooled and site-specific dietary pattern analyses were shown in **Supplementary Figure 1**.

Two sets of dietary pattern analyses were conducted: 1) pooled analysis using FFQs from all 3 sites and based on the 41 core food groups; 2) site-specific analyses using FFQs from each site and based on all food groups, including site-specific food groups. Unlike existing studies which typically uses a single approach - either a pooled dietary pattern analysis (using the same number of harmonized food groups) (2, 3) or a study-specific dietary pattern analysis (using a different number of food groups for each study) (1), we decided to use both approaches. This enables us to examine if (a) harmonizing patterns led to the loss of site-specific information and (b) if the harmonized (pooled) patterns adequately represent the populations in question by calculating Spearman correlations between the pooled and site-specific dietary pattern scores.

*Evaluation of the pooled and site-specific dietary patterns*

Each anthropometric and metabolic measure was first loge transformed to achieve an approximately normal distribution. To allow comparisons across these objective measures, these transformed values were then standardized before further analyses. Multivariable linear regression models were used to examine the associations between dietary pattern scores and each anthropometric/metabolic measure. The models were adjusted for site (only for pooled patterns), ethnicity, daily energy intakes, highest educational attainment, smoking status, parity, days of moderate and vigorous physical activity and family history of diabetes, along with mutually adjusting for other dietary patterns. In the models involving anthropometric measures (BMI and waist to hip ratio), they were mutually adjusted for in the analyses. For example, when examining the associations between dietary pattern scores and BMI, waist to hip ratio was included as a covariate. This was performed as for any given BMI, there could be differences in abdominal adiposity, which can be accounted for by adjusting for waist to hip ratio in the model (25). Additionally, studies have shown that adjusting for BMI is useful when examining the association between abdominal adiposity (measured by waist circumference) and morbidity (25).

The standardized beta estimates and their corresponding p values (denoted by asterisks) of these models were visualized using heat maps for ease of comparison across the pooled and site-specific dietary patterns. All analysis was performed using STATA 14.2 (STATACorp, Texas) and heat maps were produced using the package ggplot2 in R. Statistical tests were two sided and p values of less than 0.05 were considered to indicate statistical significance.

**Results**

Of the 1729 women recruited, 9 were excluded due to missing dietary data (n=1) or implausible daily energy intakes of less than 500 kcal/ day or more than 7000 kcal/day (n=8), leaving 1720 women for the subsequent analyses (**Supplementary Figure 2**).

*Characteristics of the participants*

The characteristics of NiPPeR participants across the three sites are shown in Table 1. Amongst the 1720 women, 46.9% were of White ethnicity, 26.7% of Chinese ethnicity and the remainder from the other three ethnic groups. 61.9% of the women had higher education qualifications, 68.0% were nulliparous and 7.3% were current smokers (**Table 1**).

*Pooled and site-specific preconception dietary patterns*

Based on the pooled analysis, three pooled dietary patterns were identified: ‘Vegetables/Fruits/Nuts’ (referred to as ‘Healthy’ subsequently), ‘Fried potatoes/Processed meat/Sweetened beverages’ (referred to as ‘Less Healthy’ subsequently) and ‘Fish/Poultry/Noodles/Rice’ (referred to as ‘Mixed’ subsequently) (**Table 2**). The pooled ‘Healthy’ pattern was characterised by higher intakes of a variety of vegetables (including salad), a variety of fruits and nuts, but lower intakes of rice and noodles/pasta (**Table 3**). The pooled ‘Less Healthy’ pattern was characterised by higher intakes of chips and fries, processed meat, sweetened beverages and white bread (**Table 4**). The pooled ‘Fish/Poultry/Noodles/Rice’ pattern was characterised by higher intakes of oily fish, white fish, poultry, leafy vegetables, eggs, noodles/ pasta and rice. The common variances explained by these three patterns were 46%, 23% and 18%, respectively.

Based on site-specific analyses, three major dietary patterns were also observed at each site (**Table 2**). For simplicity, these site-specific patterns were referred to as ‘Healthy’, ‘Less Healthy’ and ‘Mixed’ subsequently. The ‘Healthy’ patterns were ‘Vegetables/Nuts/Fruits’ in all three countries (**Table 3**). The ‘Less Healthy’ patterns were: UK ‘Processed meat/Red meat/Sweetened beverages’; SG ‘Fried foods/Processed meat/Sweetened beverages’ and NZ ‘Processed meat/Red meat/International Takeaways/Sweetened beverages’ (**Table 4**). The third pooled and site-specific patterns were collectively known as ‘Mixed’ pattern due to the heterogeneity observed across these site-specific patterns. In SG, an Asian-like diet (‘Fish/Red meat/Mushroom/Noodles’) was identified; diets made up of discretionary foods were identified in the UK (‘Pastries/cakes/Fried potatoes/Confectionery’) and NZ (‘Fried snacks/Dried/canned, citrus fruits/Fruit juices’). Factor loadings of the food groups that made up these site-specific ‘Mixed’ patterns were shown in **Supplementary Table 2**.

The pooled ‘Healthy’ and ‘Less Healthy’ pattern scores had moderate to strong correlations with the site-specific ‘Healthy’ (ρ=0.87 to 0.93) and ‘Less Healthy’ (ρ=0.65 to 0.88) dietary pattern scores, respectively (**Supplementary Table 3**). The pooled ‘Mixed’ pattern score was strongly correlated to the SG ‘Mixed’ score but correlated weakly with the UK ‘Mixed’ and NZ ‘Mixed’ scores.

*Evaluation of the pooled and site-specific dietary patterns*

Women with increasing adherence to the pooled ‘Healthy’ pattern had a higher BMI but a lower waist to hip ratio (**Figure 1**). These findings were mirrored by significant associations of increasing adherence to the UK ‘Healthy’ pattern with higher BMI and increasing adherence to the SG ‘Healthy’ pattern with a lower waist to hip ratio. No significant associations were observed between the pooled or site-specific ‘Healthy’ patterns and fasting glucose, 30 min glucose, 120 min glucose, HbA1c, HOMA-IR, hs-CRP and plasma lipids (**Supplementary Table 4**).

In contrast, women with increasing adherence to the pooled ‘Less Healthy’ pattern had a higher 30 min glucose, HOMA2-IR, LDL cholesterol and BMI (**Figure 1**). These associations were mirrored in the site-specific ‘Less Healthy’ patterns, with slight variations in standardized coefficients and statistical significance. For example, women with increasing adherence to the UK ‘Less Healthy’ pattern additionally had higher 120 min glucose and hs-CRP and those with increasing adherence to the ‘Less Healthy’ SG pattern had higher levels of fasting glucose. Notably, a significant inverse association between the ‘Less Healthy’ NZ pattern and HDL cholesterol was observed. No significant associations were observed between the pooled or site-specific ‘Less Healthy’ patterns with HbA1c, triglycerides and waist to hip ratio (**Supplementary Table 5**).

Women with increasing adherence to the pooled ‘Mixed’ pattern were likely to have higher 120 min glucose levels (**Supplementary Figure 3**). In general, the associations observed for the site-specific ‘Mixed’ patterns were unlike that of the pooled ‘Mixed’ pattern. For example, while higher adherence to the UK ‘Mixed’ pattern was significantly associated with higher HOMA2-IR and BMI, higher adherence to the SG ‘Mixed’ pattern was associated with higher fasting, 30 min glucose levels and BMI. Conversely, women with higher adherence to the NZ ‘Mixed’ pattern had significantly lower 30 min glucose levels. No significant associations between the site-specific ‘Mixed’ patterns were found for HbA1c, hs-CRP, plasma lipids and waist to hip ratio (**Supplementary Table 6**).

**Discussion**

In this multi-site study of women planning pregnancy, we identified three pooled dietary patterns (‘Healthy’, ‘Less Healthy’ and ‘Mixed’) using a harmonized approach. **Three site-specific preconception dietary patterns each in the UK, SG and NZ were identified, of which the** ‘Healthy’ and ‘Less Healthy’ **site-specific patterns were strongly correlated with the respective pooled patterns. In general, the** associations between the pooled and site-specific ‘Healthy’/ ‘Less Healthy’ patterns with objective anthropometric/metabolic measures were in the expected directions. However, we noted higher adherence to the ‘Healthy’ pooled and UK patterns with higher BMI and a lack of significant associations between the ‘Healthy’/ ‘Less Healthy’ pooled and site-specific patterns with other metabolic measures.

*Pooled and site-specific preconception dietary patterns*

**Characterized by high intakes of fruits and vegetables, the pooled ‘Healthy’ pattern of the NiPPeR study appeared similar to healthy exploratory dietary patterns among women planning pregnancy in Australia and the United Kingdom (e.g. ‘Fruit and Low-fat Dairy’ and ‘Prudent’) (26, 27). In parallel, the pooled ‘Less Healthy’ pattern of the NiPPeR study, which consisted mostly foods high in fat, sugar and refined carbohydrates (e.g. ‘Meat, High-fat & Sugar’ and ‘High-fat/sugar/takeaway’) was similarly observed among Australian, Spanish and Canadian women planning pregnancy (14, 26, 28, 29). Additionally,** the pooled ‘Healthy’ and ‘Less Healthy’ patterns were also largely similar to the site-specific ‘Healthy’ and ‘Less Healthy’ patterns, respectively. This suggests that key dietary information was retained in the pooled dietary patterns.

**Contributing higher intakes of animal protein, typical staple foods (e.g., rice and noodles/ pasta), leafy vegetables and eggs, the pooled ‘**Mixed**’ pattern in the NiPPeR study shared similarities with patterns rich in vegetables, animal protein foods (e.g. ‘Vegetables and Meat’ and ‘High-protein/fruit’) consumed by women residing in Spain, Australia and Brazil (14, 29, 30). Of note, the pooled ‘**Mixed**’ pattern in the NiPPeR study was likely driven by** the larger proportion of participants from SG, relative to UK and NZ participants. Clear differences among the ‘Mixed’ site-specific patterns were observed on closer examination. While the SG ‘Mixed’ pattern was characterised by higher intake of animal proteins, fish and a variety of vegetables, the UK and NZ ‘Mixed’ patterns had higher intakes of energy-dense foods with refined carbohydrates. In addition, the NZ ‘Mixed’ pattern was made up of food groups such as citrus fruits, fruit juices and dried or canned fruits. These site-specific consumption patterns have been previously reported in other women of comparable age to the women enrolled in the NiPPeR study (UK: ‘Snacking’ (31), ‘Sugary foods, dairy’ (32) and NZ: ‘Refined and processed’, ‘Sweet and savoury snacking’ (33)), reflecting the cultural and regional differences in intakes of specific foods.

*Evaluation of the pooled and site-specific dietary patterns*

**Consistent with several studies examining the associations between exploratory dietary patterns and anthropometric measures (9-11), women with higher adherence to the pooled and site-specific ‘Healthy’ patterns had a lower waist to hip ratio. Conversely, those with higher adherence to the pooled and site-specific ‘Less Healthy’ patterns had a higher BMI. Contrary to expectations, increasing adherence to the pooled and UK ‘Healthy’ patterns were associated with higher BMI. It is possible that intakes of energy-dense potatoes/starchy vegetables of the pooled ‘Healthy’ pattern and intakes of dried/canned fruits and frying fats/oils of the UK ‘Healthy’ pattern contributed to weight gain and hence a higher BMI observed (34, 35). With respect to the metabolic measures, women with higher adherence to the pooled and site-specific ‘Less Healthy’ patterns had less favorable plasma glucose and lipid profiles and higher levels of hs-CRP, which has been reported by previous studies (8, 11). Women with higher adherence to these ‘Less Healthy’ patterns generally have higher intakes of refined grains, sugary foods and drinks and fried foods high in saturated and trans fat, contributing to poorer glycemic, insulinemic response and higher levels of inflammation observed (8).**

**In contrast, differing associations were observed for the pooled and site-specific ‘Mixed’ patterns, which are largely attributed to differences among these dietary patterns as aforementioned. While the UK and SG ‘Mixed’ patterns were associated with less favourable metabolic profiles (higher BMI, higher insulin resistance, fasting and 30 min glucose), the NZ ‘Mixed’ was associated with lower 30 min glucose levels. This may reflect intakes of beans and legumes as part of the NZ ‘Mixed’ pattern that could have enhanced the glycemic and insulinemic response (36). Future diet-related investigations might explore whether these pooled and site-specific ‘Mixed’ patterns play a role in subsequent maternal and child health outcomes.**

**Collectively known as metabolic risk biomarkers, the objective** anthropometric/metabolic **measures examined in this study are typically used to predict risk of chronic cardio-metabolic diseases among individuals (37). Given this, the associations observed for the ‘Less Healthy’ patterns were not unexpected as higher adherence to suboptimal diets were known to be associated with increased cardio-metabolic risks (8). Taken together, the associations between the pooled ‘Healthy’/ ‘Less Healthy’ patterns were generally in the expected directions.**

**Strengths and limitations**

Strengths of our study include combining previous harmonization methods to examine preconception diets and using multiple objective anthropometric/metabolic measures to strengthen our findings. Additionally, this study adds to the growing evidence on the overall diets of women planning pregnancy. However, this study was limited in several ways.

First, self-reported food intakes, measured using a FFQ, are prone to overestimation, as observed by several studies (38, 39). However, FFQs have been found to be useful in ranking participants’ dietary intakes and are commonly used to examine habitual dietary intakes and their associations with health outcomes (40, 41).

Second, due to the cross-sectional nature of this study, we were unable to ascertain temporal associations between preconception dietary patterns and the objective anthropometric/metabolic measures. Nevertheless, except BMI, the associations observed were in the expected directions and were consistent with previous studies. However, reverse causation could have occurred as approximately half of the participants in the NiPPeR study were either overweight or obese. Women with higher BMI could have consumed healthier diets to lose weight during the preconception period. For others who did not change their usual diets, it is expected that higher adherence to ‘Less healthy’ diets were associated with less favourable metabolic risk biomarker profiles (e.g. higher levels of hs-CRP).

Third, the NiPPeR study was not designed to recruit representative samples from each country. Despite this, the results presented here provide valuable insights into preconception dietary patterns across the three sites.

Fourth, information on the degree of pregnancy planning, which can be assessed using the London Measure of Unplanned Pregnancy (LMUP) (42), was not collected in this study. Women with a higher degree of pregnancy planning could have higher adherence to ‘Healthy’ dietary patterns and thus have a more favourable anthropometric/metabolic profile.

Fifth, we noted that several food groups loaded in more than one dietary pattern. This is not unusual given the complexity of dietary intakes and highlights the importance of examining dietary patterns that consists of multiple food groups instead of single food groups in isolation. However, the cross-loading of foods on different patterns suggests that these dietary patterns should ideally be re-generated for the analytic sample of interest to fully represent their existing consumption patterns. Future studies could consider using diet indices, generated from a pre-defined list of foods and beverages, to complement findings from dietary patterns.

**Conclusion and future research**

Despite differences in country of residence and ethnicity, similar preconception ‘Healthy’ and ‘Less Healthy’ pooled and site-specific dietary patterns were identified. In general, these patterns had expected associations with objective anthropometric/metabolic measures, providing a basis for **future diet-related investigations.** **However, future studies involving similar populations are required to confirm these findings.**

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**Data Availability Statement**: A Trial Consultative Panel, comprising senior representatives from the academic institutions undertaking the study and the industry partner, has been set up and will consider associated studies requesting access to data and materials. The data underlying this article will be shared on reasonable request to the corresponding author.

**Ethics approval** After independent, full, external peer review, the study protocol and subsequent amendments have been approved by the Research Ethics Committees at each of the three study sites (Southampton: Health Research Authority NRES Committee South Central Research Ethics Committee (REC), reference 15/SC/0142), the Health and Disability Ethics Committee (HDEC) (New Zealand) reference 15/NTA/21 and the National Healthcare Group Domain Specific Review Board (NHG DSRB) (Singapore) reference 2015/ 00205). The trial is an academic-led study registered at ClinicalTrials.gov (NCT02509988), Universal Trial Number U1111-1171-8056. It has received intramural/infrastructure funding support at each of the three sites (UK Medical Research Council (MC\_UU\_12011/4); Singapore National Medical Research Council (NMRC/TCR/012-NUHS/2014); Gravida (National Centre for Growth and Development, New Zealand, no reference number)), with cofunding from Nestec Ltd. (RDCU000485), who have formulated the trial intervention. Information Sheets are provided to potential participants ahead of them being approached for consent by research staff. This study is being conducted in compliance with the protocol, Good Clinical Practice and the applicable regulatory requirements.

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**Figure 1**: **Visual representation of the cross sectional associations between the pooled and site-specific ‘Healthy’ and ‘Less Healthy’ dietary pattern scores with objective anthropometric and metabolic measures.** Cells with asterisks indicate that the standardized beta coefficients were statistically significant, where p < 0.001 (\*\*\*), p < 0.01 (\*\*) and p < 0.05 (\*).

**Table 1**: Characteristics of 1720 women planning pregnancy in the NiPPeR cohort at baseline

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **All** | **UK** | **SG** | **NZ** |
| Number of women | 1720 | 460 | 660 | 600 |
| Age ± SD (years) b | 31 ± 4 | 30 ± 4 | 31 ± 4 | 31 ± 4 |
| BMI ± SD (kg/m2) b | 26 ± 6 | 27 ± 6 | 24 ± 6 | 27 ± 7 |
| Overweight/ Obese (%) | 51.7 | 55.7 | 50.0 | 50.4 |
| Ethnic origin (%) |
| White Caucasian | 46.9 | 93.9 | - | 62.5 |
| Chinese | 26.7 | 0.2 | 63.0 | 7.0 |
| South Asian (Indian, Pakistani, Bangladeshi) | 7.0 | 2.4 | 8.3 | 9.2 |
| Malay | 9.5 | 0.2 | 24.7 | - |
| Other (Polynesians, Blacks and other Asians) | 9.8 | 3.3 | 3.9 | 21.3 |
| Nulliparous (%) | 68.0 | 65.4 | 65.9 | 72.2 |
| Bachelor's degree and above (%) | 61.9 | 50.9 | 60.3 | 72.0 |
| Household income quintiles (%) |
| Q1 + Q2 (lowest income bracket) | 17.4 | 7.1 | 32.0 | 8.9 |
| Q3 | 22.0 | 15.4 | 28.8 | 19.7 |
| Q4 + Q5 (highest income bracket) | 60.6 | 77.5 | 39.2 | 71.5 |
| Current smoker (%) | 7.3 | 9.8 | 6.8 | 5.8 |
| No alcohol consumption in past 3 months (%) | 31.1 | 15.9 | 53.8 | 17.8 |
| Instances of moderate/vigorous physical activity in the past week (days/week) c | 3 (2, 5) | 3 (1, 5) | 2 (1, 5) | 4 (2, 6) |
| Daily total screen time in the past week (hours) c | 3 (2, 4) | 3 (2, 4) | 3 (2, 4) | 3 (2, 4) |
| Daily total sitting time in the past week (hours) c | 7 (3, 8) | 6 (3, 8) | 7 (4, 9) | 6 (4, 8) |
| Estimated daily energy intake (kcal) c | 1955 (1593, 2425) | 1853 (1570, 2221) | 1850 (1476, 2339) | 2158 (1788, 2671) |
| a Missing values for BMI (n=3), overweight/obese were based on Asian BMI cut-offs for SG participants and non-Asian BMI cut-offs for UK and NZ participants. For Asians (including Chinese, Indians, Pakistani, Bangladeshi, Malay, mixed Asian), BMI ≥ 23 to < 27.5 kg/m2 was defined as overweight and BMI ≥ 27.5 kg/m2 was defined as obese. For non-Asians (including White Caucasian, Polynesian, Black, mixed Asian-non-Asian), BMI ≥ 25 to < 30 kg/m2 was defined as overweight and ≥ 30 kg/m2 was defined as obese; Household income quintiles (n=117); Current smoker (n=4); Instances of moderate/vigorous physical activity (n=10); Daily total screen time (n=17); Daily total sitting time (n=12) |
| b Values presented are mean ± SD (standard deviation) |
| c Values presented are median (25th percentile, 75th percentile) |

**Table 2**: Characteristics of pooled and site-specific dietary patterns in the NiPPeR cohort

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Pooled analysis a** | **UK b** | **SG b** | **NZ b** |
| n | 1720 | 460 | 660 | 600 |
| Number of food groups | 41 | 51 | 47 | 53 |
| Number of factors | 3 | 3 | 3 | 3 |
| Sum of common variance explained by 3 factors (%) | 87 | 52 | 69 | 56 |
| **Dietary patterns identified** |
| ‘Healthy’ pattern | Vegetables/Fruits/Nuts | Vegetables/Nuts/Fruits | Vegetables/Nuts/Fruits | Vegetables/Nuts/Fruits |
| ‘Less Healthy’ pattern | Fried potatoes/Processed meat/Sweetened beverages | Processed meat/Red meat/Sweetened beverages | Fried foods/Processed meat/Sweetened beverages | Processed meat/Red meat/International Takeaways/Sweetened beverages |
| ‘Mixed’ pattern | Fish/Poultry/Noodles/Rice | Pastries/cakes/Fried potatoes/Confectionery | Fish/Red meat, Mushroom/Noodles | Fried snacks/Dried/canned/citrus fruits/Fruit juices |
| a Pooled analysis includes participants from UK, SG and NZ. 41 core food groups that included only foods common to all three countries were used for deriving dietary patterns |
| b Site-specific food groups were added to the analysis on top of the 41 core food groups, resulting in 51, 47 and 53 food groups for UK, SG and NZ |

**Table 3**: Factor loadings of food groups of the ‘Healthy’ pooled and site-specific patterns

|  |  |
| --- | --- |
|  | **‘Healthy’ patterns** |
|  | **Pooled** | **UK** | **SG** | **NZ** |
| Common variance explained (%) | 46 | 24 | 25 | 19 |
| **Food groups** |  |  |  |  |
| Salad | 0.58 | 0.31 | 0.35 | 0.59 |
| Root vegetables | 0.56 | 0.47 | 0.53 | 0.52 |
| Peas, green beans, legumes and pulses | 0.55 | 0.50 | 0.55 | 0.32 |
| Other vegetables and gourds | 0.52 | 0.49 | 0.39 | 0.52 |
| Tomatoes | 0.50 | 0.45 | 0.58 | 0.34 |
| Bananas | 0.49 |  | 0.39 |  |
| Potatoes and starchy vegetables | 0.46 |  | 0.42 | 0.34 |
| Yoghurt | 0.43 | 0.29 | 0.29 |  |
| Cheese | 0.42 |  |  | 0.26 |
| Apples and pears | 0.42 | 0.40 | 0.38 |  |
| Grapes, berries, stone fruits and tropical fruits | 0.41 | 0.39 | 0.38 |  |
| Nuts | 0.38 | 0.40 | 0.46 | 0.35 |
| Breakfast cereals | 0.38 |  | 0.27 |  |
| Citrus fruits and fruit juices | 0.38 |  | 0.34 |  |
| Leafy vegetables | 0.34 | 0.68 | 0.48 | 0.57 |
| Hot beverages | 0.34 |  |  |  |
| Dried and canned fruits | 0.25 | 0.34 | 0.24 |  |
| Noodles and Pasta | -0.27 |  |  |  |
| Rice | -0.42 |  |  |  |
| White bread |  | -0.30 |  |  |
| Wholemeal/multigrain/brown bread | 0.36 |  |  |  |
| Eggs |  | 0.44 |  | 0.34 |
| Oily fish, white fish, shellfish and other seafood | 0.35 |  | 0.26 |  |
| Onions (UK and NZ only) | 0.46 |  | 0.26 |  |
| Frying fats and oils (UK and NZ only) | 0.31 |  |  |  |
| Cream (UK and NZ only) | 0.31 |  |  |  |
| Gravy, stock and seasonings (UK and NZ only) | 0.30 |  |  |  |
| Mushroom (UK and SG only) | 0.28 |  |  |  |
| Steamed snacks / dim sum / ethnic bread (SG and NZ only) | 0.33 |  |  |  |
| Avocado (NZ only) |  |  |  | 0.25 |
| Water (NZ only) |  |  |  | 0.25 |
| a Values are correlation coefficients between each food variable and the dietary pattern.b For simplicity, only food groups with absolute values of at least 0.25 were listed |

**Table 4**: Factor loadings of food groups of the ‘Less Healthy’ pooled and site-specific patterns

|  |  |
| --- | --- |
|  | **‘Less Healthy’ patterns** |
|  | **Pooled** | **UK** | **SG** | **NZ** |
| Common variance explained (%) | 23 | 15 | 23 | 22 |
| **Food groups** |  |  |  |  |
| Chips and fries | 0.48 |  | 0.55 | 0.52 |
| Crisps and savoury snacks | 0.45 | 0.36 | 0.41 | 0.35 |
| Ham, bacon, sausage and other processed meat | 0.43 | 0.59 | 0.54 | 0.55 |
| Sweetened beverages | 0.37 | 0.27 | 0.36 | 0.36 |
| Pastries and cakes | 0.36 |  | 0.51 |  |
| Chocolate | 0.35 | 0.29 | 0.35 |  |
| White bread | 0.34 |  | 0.33 | 0.46 |
| Pizza | 0.33 |  | 0.33 | 0.50 |
| Other meats (pork, lamb, beef) | 0.31 | 0.43 |  | 0.53 |
| Sweet biscuits and cookies | 0.29 | 0.34 |  | 0.29 |
| Potatoes and starchy vegetables | 0.28 |  |  | 0.37 |
| Poultry |  | 0.34 |  | 0.36 |
| Salad |  | 0.33 |  |  |
| Diet drinks |  | 0.25 |  |  |
| Other vegetables and gourds | -0.33 |  |  |  |
| Tofu/beancurd/vegetarian foods | -0.46 |  |  |  |
| Sweet and savoury spreads | 0.34 |  |  |  |
| Ice cream |  |  | 0.34 |  |
| Savoury biscuits and crackers | 0.31 |  |  |  |
| Sweet biscuits and cookies | 0.30 |  |  |  |
| Cheese |  |  | 0.27 |  |
| Noodles and Pasta |  |  | 0.45 |  |
| Oily fish, white fish, shellfish and other seafood | 0.35 |  |  |  |
| Buns |  |  |  | 0.30 |
| Liver and offal (UK and SG only) | 0.35 |  |  |  |
| Fried snacks/ dim sum/ ethnic bread (SG and NZ only) | 0.57 |  |  |  |
| Steamed snacks / dim sum / ethnic bread (SG and NZ only) | 0.32 | 0.25 |  |  |
| International Takeaways (NZ only) |  | 0.40 |  |  |
| Sweets/candies (UK and NZ only) |  | 0.27 |  |  |
| a Values are correlation coefficients between each food variable and the dietary pattern.  |
| b For simplicity, only food groups with absolute values of at least 0.25 were listed |