Regular Article

Diet quality trajectories and cardiovascular phenotypes/metabolic syndrome risk by 11-12 years

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Abbreviations: LSAC: Longitudinal Study of Australian Children; bpm: beats per minute; CI: confidence interval; mmHg: millimetre of mercury; CVD: cardiovascular disease; PWV: pulse wave velocity; MetS: metabolic syndrome; BMI: body mass index; IMT: intima-media thickness; SEP: socioeconomic position; CV: cardiovascular; SD: standard deviation; SMD: standard mean difference; DSS: Department of Social Services; AIFS: Australian Institute of Family Studies; ABS: Australian Bureau of Statistics; SBP: systolic blood pressure; DBP: diastolic blood pressure.

ABSTRACT

Objective: To investigate associations between early-life diet trajectories and preclinical cardiovascular phenotypes and metabolic risk by age 12 years.

Methods: Participants were 1 861 children (51% male) from the Longitudinal Study of Australian Children. *At five biennial waves from 2-3 to 10-11 years:* Every 2 years from 2006 to 2014, diet quality scores were collected from brief 24-hour parent/self-reported dietary recalls and then classified using group-based trajectory modelling as 'never healthy' (7%), 'becoming less healthy' (17%), 'moderately healthy' (21%) and 'always healthy' (56%). *At 11-12 years:* During children's ~~1.5 h to 3.5 h~~ physical health Child Health CheckPoint (2015 to 2016) we measured cardiovascular functional (resting heart rate, blood pressure, pulse wave velocity, carotid elasticity/distensibility) and structural (carotid intima-media thickness, retinal microvasculature) phenotypes; and metabolic risk score (composite of body mass index z-score, systolic blood pressure, high-density lipoproteins cholesterol, triglycerides and glucose). Associations were estimated using linear regression models (n = 1 100 to 1 800) adjusted for age, sex and socioeconomic position.

Results: Compared to 'always healthy', the 'never healthy' trajectory had higher resting heart rate (2.6 bpm, 95% CI 0.4, 4.7) and metabolic risk score (0.23, 95% CI 0.01, 0.45), and lower arterial elasticity (-0.3% per 10mmHg, 95% CI -0.6, -0.1) and distensibility (-1.2%, 95% CI -1.9, -0.5) (all effect sizes 0.3 to 0.4). Heart rate, distensibility and diastolic blood pressure were progressively poorer for less healthy diet trajectories (linear trends $p \leq 0.02$). Effects for systolic blood pressure, pulse wave velocity and structural phenotypes were less evident.

Conclusions: Children following the least healthy diet trajectory had poorer functional cardiovascular phenotypes and metabolic syndrome risk, including higher resting heart rate, one of the strongest precursors of all-cause mortality. Structural phenotypes were not

associated with diet trajectories, suggesting the window to prevent permanent changes remains open to at least late childhood.

INTRODUCTION

The Global Burden of Disease Study estimates that in 2017 dietary risk factors accounted for 11 million deaths and 255 million disability-adjusted life years, led by cardiovascular disease (CVD).[1] CVD risk develops across the life course[2] and, consistent with a life course accumulative model,[3] childhood diet quality may influence emerging cardiovascular (CV) and metabolic phenotypes and be important to adult CVD. If such impacts are already evident in childhood, then motivation for public health promotion to improve poor diet quality very early in the life course will be heightened.

Child and adolescent consumption of specific foods and food groups has repeatedly been linked with CVD risk factors. For example, higher long-chain omega-3 polyunsaturated fatty acid, fish and/or dairy food consumption have been associated with healthier adolescent microvasculature (wider retinal arterioles, narrower venules); with soft drinks and carbohydrate nutrition showing the reverse.[4-6] When considering overall diet using questionnaire-derived scores, the few null studies are outweighed by studies indicating that less healthy diet scores predict poorer risk profiles.[7-13] For example, children and adolescents with high consumption of fruits, vegetables, wholegrains and low consumption of total fat, saturated fat, cholesterol, sodium (the DASH diet) have lower blood pressure and reduced incidence of metabolic syndrome.[9, 10, 12]

Several large longitudinal studies (e.g. The Avon Longitudinal Study of Parents and Children (ALSPAC); The Cardiovascular Risk in Young Finns Study) also demonstrate prospective associations between poor childhood diet quality and CVD risk factors in later childhood, adolescence, and adulthood.[14-17] Most recently, ALSPAC investigators demonstrated that distinct eating behavior trajectories (e.g. overeating, fussy eating) throughout children's first decade associate with their Body Mass Index (BMI) at age 11 years.[18] Specifically, compared to children following a low and stable trajectory of overeating

from age 15 months to 10 years, children following an early and increasing trajectory of overeating recorded much greater BMI at the age 11 follow up.[18] From a public health perspective, trajectory analyses are more appealing than individual-level longitudinal analyses because one can pinpoint which typical trajectories of behavior are best (and worst) for population level health outcomes.[19]

However, longitudinal studies employing diet *quality* scores are scarce. In those that exist, diet scores are often only measured at baseline or the focus centres on mean scores over time, follow-up is usually short (e.g. commonly 3-4 years),[8, 12, 13] and piecemeal examination of isolated exposures and phenotypic outcomes precludes a more complete understanding of lifetime diet quality and emerging cardiometabolic phenotypes. In our small community sample (n=188), children following a consistently poor vs healthy diet trajectory from age 4-15 years had a resting heart rate 11 beats per minute faster at age 15 years[20] – an indicator highly predictive of all-cause mortality.[21] However, few large cohort studies have repeated measurements of childhood diet quality over such a lengthy time-span.

Here, we report on a much larger national cohort with previously-derived diet trajectories from ages 2-11 years[22] and extensive phenotypic cardiovascular measures at age 11-12 years.[23] Specifically, we aimed to determine the extent to which childhood diet trajectories across the first decade of life are already associated with cardiovascular functional and structural phenotypes and metabolic syndrome risk by age 11-12. We expected the strongest relationships to emerge between the diet trajectories and vascular functional and metabolic changes, which usually manifest before structural changes.

METHOD

Participants & Procedure: Dietary exposure data were collected within the Birth (B) cohort of the nationally-representative Longitudinal Study of Australian Children (LSAC).[24] In

2004, infants aged 0-1 years were sampled from Australia's universal Medicare healthcare database using a two-stage clustered random sampling design[24] and then followed biennially. Of the original 5 107 0-1-year olds (57% uptake), 3 764 (74%) were retained to Wave 6 in 2014 at age 10-11 years. Cardiometabolic phenotypic outcomes relevant to non-communicable diseases (NCD) were measured at age 11-12 years within the Child Health CheckPoint (CheckPoint), LSAC's physical and biomarkers module nested between LSAC Waves 6 and 7.[23] Of the LSAC families retained to Wave 6, 3 513 consented to be contacted for the CheckPoint, and 1 874 (53%) families ultimately participated across Australia (Supplementary Figure 1).

The CheckPoint ran from February 2015 to March 2016, offering child-parent dyads a visit to its full Main Assessment Center (n=1 356) in one of Australia's 7 largest cities (mostly state capitals), a condensed Mini Assessment Center (n=153) visit in 8 regional towns, or a shorter home visit (n=365). CheckPoint methods (described elsewhere[23]) comprised a comprehensive 1.5 to 3.5 hour assessment, divided into 15-minute physical health assessment "stations" that participants rotated through in a set sequence. Relevant to this study, stations included "Heart Lab", "See Here", "Measure Up" and "Young Bloods", where trained researchers measured cardiovascular parameters and body composition, and took semi-fasting peripheral blood samples.

CheckPoint protocols were approved by The Royal Children's Hospital (Melbourne, Australia) Human Research Ethics Committee (33225D) and the Australian Institute of Family Studies Ethics Committee (14-26), which also approved LSAC. The attending parent provided written informed consent for their own and their child's participation.

Dietary exposure measure: As detailed in Table 1, every 2 years at each of Waves 2-6 (ages 2-3 to 10-11 years), dietary data were collected via computer-assisted self-interviews in the

format of a food diary on the frequency of the child's intake of 12-16 items over the previous 24 hours or yesterday, transitioning from parent-proxy to child self-report from age 10 years.[22] Supplementary Figure 2 provides an example food diary. Food items focused on frequency (e.g. not at all, once, more than once), not the amount or serving size, of fruit, vegetables, water, fatty foods, sugary foods, sweetened drinks, milk products or alternatives. Using Australian Dietary Guidelines and other resources, we have previously calculated diet quality scores at each wave.[22] For each category of food, we assigned a score depending on whether frequency of consumption did not meet (0), partially met (1) or fully met (2) the associated dietary guideline. Total scores ranged from 0-14, with 14 being the healthiest.

As previously published,[22] we conducted group-based trajectory modelling ('traj' plug-in in Stata/IC version 14.2[25]) to classify longitudinal diet trajectories from ages 2-3 to 10-11 years (LSAC Waves 2-6). Children's diet quality score from each wave was specified as the dependent variable (to be summarized in the trajectory) and age at each wave as the independent (explanatory) variable. Children needed at least two diet scores across Waves 2 to 6 to be included (88.2% of original sample). We fitted models with one to eight trajectories, and removed non-significant ($p<.05$) quadratic or cubic parameters until a model contained no non-significant parameters.[26] We aimed to maximise model fit on the basis of Bayesian criterion values and the log Bayes Factor, and for each trajectory to contain enough children to support further analyses. A model including four trajectories was selected with children assigned to the trajectory for which their probability of membership was highest. The resulting trajectories demonstrate expected gradients with socioeconomic determinants[27] and parental health behaviours,[28] and replicate in an older cohort of children and adolescents.[22]

Although we hypothesize that the overall lifelong diet trajectory is of most importance to the developing phenotype, logically the quality of diet measures most closely in time to the

phenotypic outcomes would have the strongest point association. Therefore, *a priori*, to examine trends in outcomes according to worsening diet quality, we ordered trajectories from most to least healthy as at Wave 6 rather than an earlier Wave, as follows (see Figure 1, reproduced[22]):

(1) always healthy (56% of the analytic sample),

(2) moderately healthy (21%),

(3) becoming less healthy (17%),

(4) never healthy (7%).

Cardiometabolic outcome measures

Preclinical vascular phenotypes: We chose measures that are widely used in assessing adult CVD and relevant to pediatric populations.[29] Table 1 outlines each protocol and measurement, with standalone methods available in our methodological publications.[23, 30-33] Note that sample sizes differ between the eight outcome measures because of equipment or logistical constraints at different testing sites (i.e. Main vs. Mini Assessment Centres vs. home visits) and due to missing data on some measures (e.g. bloods, retinal photography). We list the sample size for each outcome measure in Supplementary Figure 1.

Resting heart rate, blood pressure and carotid-femoral pulse wave velocity (PWV) were captured using SphygmoCor XCEL (AtCor Medical, Sydney, Australia) at both Main and Mini Assessment Centres and at home visits after participants had several minutes rest. Carotid artery elasticity, distensibility and intima-media thickness were available only for children who attended a Main or Mini Assessment Center. These were measured following PWV, using standardized carotid artery ultrasound protocols with a portable ultrasound machine and 10MHz linear array probe (Vivid-I, GE Healthcare, Chicago, Illinois, USA) and scored with a semiautomatic edge-detection software program (Carotid Analyser, Medical

Imaging Applications, Coralville, IA, USA). For those attending a Main Assessment Center, optic disc-centred retinal photographs were obtained without mydriasis using a fundus camera (EOS 60D SLR). Images were scored using IVAN software (University of Wisconsin, Madison, USA) to estimate retinal vascular caliber.

Metabolic syndrome risk (MetS): Semi-fasting (median 4.2 hours post-prandial) peripheral blood was collected at Main and Mini Assessment Centres and processed onsite within 4 hours. Serum total triglycerides, total cholesterol, high-density lipoprotein cholesterol, and glucose were quantified with high-throughput proton nuclear magnetic resonance spectrometry (AVANCE III 500 MHz spectrometer; Bruker Corporation, Billerica, MA). Z-scores were calculated[34] from a formula derived from the National Health and Nutrition Examination Survey (12-19 year olds), drawing on BMI z-score, systolic blood pressure, high-density lipoprotein cholesterol, triglycerides and glucose.[35]

Adverse phenotypes are represented by higher resting heart rate, blood pressure and/or metabolic syndrome risk score; greater arterial stiffness (quicker/higher pulse wave velocity, less arterial elasticity, less distensibility); increased carotid intima-media thickness; and narrower arteriolar and/or wider venular vessels.

Statistical Analyses: Analyses were conducted with Stata/IC version 14.2, using the *svy* package to account for multi-level sampling by postcode and to apply survey weights adjusting for non-response and loss to follow up from LSAC Wave 1.[36] Participants were included if they had sufficient measures to be allocated to their diet trajectory group (i.e. ≥2 diet scores, 88.2% of original sample) and at least one outcome measure (n=1 861; see Supplementary Figure 1). *A priori* potential confounders were Wave 1 socioeconomic position (SEP, a composite LSAC-derived z-score) and sex, and age at CheckPoint assessment.

In adjusted linear regression analyses, diet trajectories were entered as four categorical predictors (reference: always healthy) and CV phenotypes as continuous outcomes. We then examined linear trends by entering the categorical trajectory variables (ordered as above) into adjusted linear regression analyses to examine the trajectory's strengths of association with the outcome. We did not formally adjust for multiple testing because our priority was to interpret replicable, rather than isolated, patterns within the data – to which p-values are a minor contributor.[37]

Sensitivity analyses were conducted: (1) adjusted for BMI z-score (and puberty) in all models (except MetS), and for blood pressure in models for PWV, IMT, distensibility and microvasculature (Supplementary Table 2); (2) adjusted for physical activity and fitness, which, although potential confounders, were not included in the main models due to missing data (Supplementary Table 3); (3) stratified by sex (Supplementary Table 4); and (4) to be sure that the variance in our main models was not explained by concurrent diet quality, replaced the diet trajectory variables (Waves 2-6) with children's most recent diet quality score at Wave 6 (Supplementary Table 5).

RESULTS

The mean age of the sample (n=1 861) was 11.5 years (SD 0.5), and girls (49%) and boys (51%) were roughly equally represented; mean BMI z-score (0.3, SD 1.0) was above historical norms, aligning with national data.[38] The mean family SEP was 0.32 SD above the mean SEP of all families at LSAC Wave 1.[39]

Main Analyses: Table 2 presents the adjusted associations between diet trajectories and CV outcomes. Children who followed the 'never healthy' diet trajectory showed worse resting heart rate, carotid artery elasticity and distensibility and metabolic syndrome risk than those

in the 'always healthy' trajectory (all effect sizes 0.3 to 0.4 standardized mean difference (SMD)). Their heart rate was on average 2.6 bpm faster (95% CI 0.4 to 4.7), arterial elasticity 0.3% per 10mmHg lower (95% CI -0.6 to -0.1), distensibility 1.2% lower (95% CI -1.9 to -0.5) and metabolic syndrome risk score 0.23 units higher (95% CI 0.01 to 0.45). Findings for blood pressure, pulse wave velocity and the structural large (carotid IMT) and small (retinal arteriolar and venular) vessels were less evident, with effect sizes of 0.1 to 0.2 (Table 2). All but retinal venular effects were in the hypothesized direction (i.e. poorer scores for 'never healthy' diet trajectory).

Examining the trend across the series of worsening diet trajectories (from always healthy, to moderately healthy, to becoming less healthy, to never healthy), Table 3 shows that resting heart rate (p=.02), distensibility (p=.01) and diastolic blood pressure (p=.02) all deteriorated as diet quality worsened. Across poorer diet trajectories, heart rate rose by 0.6 bpm (95% CI 0.1 to 1.2, SMD 0.07) per category, diastolic blood pressure rose by 0.4 mmHg (95% CI 0.1 to 0.8, SMD 0.07), and distensibility dropped by 0.3% (95% CI -0.5 to -0.1, SMD -0.08). In Figure 2 we illustrate the standardized effect sizes (i.e. mean differences) across the trajectories for all functional phenotypic measures from Table 2. Trends for all other outcome variables were similar but, aside from systolic blood pressure (p=.09), were very weak (p>.15; SMD<0.05, Table 3).

Sensitivity Analyses: All main conclusions remained essentially unchanged in sensitivity analyses adjusting for puberty, BMI z-score and blood pressure (where appropriate) in the first instance (Supplementary Table 2), and then fitness and physical activity (Supplementary Table 3). However, some effects weakened for the linear trend analyses (Supplementary Tables 2 and 3). When stratified by sex, most effects for the functional artery measurements and metabolic syndrome risk were higher amongst girls and lower for boys (Supplementary

Table 4). Last, to ensure that the variance explained in our main models was not accounted for by concurrent diet quality, we replaced the longitudinal childhood diet trajectory exposure (Waves 2-6) with Wave 6 diet quality score alone. As expected, all effect sizes reduced substantially in this analysis (Supplementary Table 5).

DISCUSSION

Statement of principal findings: In this large population-based cohort of 11-12-year-old children, those who had consistently followed a 'never healthy' diet trajectory since toddlerhood demonstrated worse cardiovascular function (higher resting heart rate, lower carotid artery elasticity and distensibility) and poorer metabolic health than children consistently following a 'healthy' diet. If causal, the size of these effects (0.3 to 0.4 SMD) would likely be important at the population level. Adverse effects for blood pressure, pulse wave velocity and structural changes of the large and small arteries (carotid intima-media thickness, retinal arterioles) were less evident. Most findings were robust in sensitivity analyses and strongest in girls, and they were not explained by BMI or concurrent diet exposure.

Strengths and limitations: Our study is strengthened by its longitudinal design, repeated diet quality reporting throughout the whole of childhood, and the breadth of objective outcome measures. The positive (heart rate, vascular stiffness) and null findings (structural phenotypes) were congruent with our earlier preliminary analysis in a smaller cohort.[20] This replication suggests that these relationships may be generalizable and not chance findings. While parent and self-reported diet quality measures are subject to measurement error, the use of latent variables across multiple time points more reliably identifies patterns over time.[40] As supported by a recent systematic review,[41] we sought to capture and interpret

overall behavioral dietary patterns rather than quantity of individual foods or nutrients. However, there remains an urgent unmet need for accurate and objective diet tools suited to repeated measurements in population research. Furthermore, given the high percentage of children following the healthiest trajectory and low percentage of children following the unhealthiest trajectory, we acknowledge that parents (and children) in our cohort may have reported, or their child may have been following, a healthier diet than is reported in other Australian cohorts.[42, 43] Together with our loss to follow up (Supplementary Figure 1), these limitations imply that findings should be cautiously generalized to the population. It is also noteworthy that children from disadvantaged families are more likely to have poor diets,[43] have at-risk vascular phenotypes,[44] and later in their life course be more likely to develop CVD.[45] Because these children were under-represented in this cohort, we may have underestimated true cardiovascular and metabolic differences between trajectories for this group and the population as a whole. Last, with the intent to best capture children's evolving phenotype, we included multiple outcomes, many of which contained missing data points due to equipment or logistical constraints. It is therefore possible that some of our results are chance findings, but given the consistent patterns obtained across outcome measures and cohorts,[20] we are confident that our results are meaningful.

Comparison with prior literature: The recent Global Burden of Disease Study confirmed that a suboptimal diet during adulthood is associated with a massively higher burden of NCDs worldwide.[1] For CVD, our results suggest that this burden may begin in childhood, even before adolescence. The direction of obtained effects is in line with our own[20] and other previous studies examining child and adolescent diet quality scores and phenotypic outcomes.[7-13] However, past research has obtained positive associations between dietary measures (though not trajectories) and some phenotypic measures for which we obtained

small or null associations (e.g. blood pressure, retinal microvasculature). It is conceivable that methodological differences between ours and past studies are too great to draw meaningful comparisons, as we employed a data reduction technique (i.e. group-based trajectory modelling) to answer a different 'life-long' research question. That is, we cannot generalize or make direct comparisons with the majority of past literature because many such studies were cross-sectional and/or examined different research questions using isolated nutrients or food groups (e.g. sugar-sweetened beverages, dairy foods), rather than decade-long diet quality scores.[4-6] Future research may look at developing trajectories for these food groups, rather than using trajectories of whole diet scores. It is conceivable that lifetime consumption of particular foods or nutrients has distinct effects on different aspects of children's developing phenotype that we did not detect.[46]

Implications: Overall, the small linear trends across worsening diet trajectories (statistically significant or not) may signal higher risk for poor cardiovascular and metabolic health later in the lifecourse.[47] Even though the sample means for resting heart rate are well within the normal range,[48, 49] the differences between the 'always healthy' (M=73.8 bpm, 25th percentile) and 'never healthy' (M=76.4 bpm, >50th percentile) groups are substantive. In the UK's National Child Development Study, heart rate was one of the strongest predictors of all-cause mortality among mid-life adults, outperforming more traditional markers such as triglycerides.[21] In midlife, every additional 5 bpm above a heart rate of 60 has been shown to increase mortality risk by around 12% over 28-year follow up.[50] Metabolic syndrome in adulthood predicts type 2 diabetes, premature CVD and all-cause mortality, and having even one or two MetS risk factors doubles the risk of CVD mortality.[51] Similar risks are associated with decreased carotid artery elasticity and distensibility.[52, 53]

Functional impairment of the arterial wall (such as vascular stiffness) becomes evident early in the atherosclerotic process, before structural wall changes and clinical symptoms of CVD develop. Consistent with this, we saw larger effect sizes with unhealthy diet trajectories of 0.3 to 0.4 for the functional outcomes than for the 0.1 to 0.2 SMDs toward narrower arterioles and increased IMT. Functional changes often track from childhood to adulthood,[54] triggering sympathetic over-activity or increased cardiac stress[55] and go on to predict structural organ damage, and cardiovascular and all-cause mortality. If these diet trajectories and preclinical phenotypic changes track through the lifecourse,[54] the population health implications could be considerable, especially if structural phenotypic changes also develop. Our absence of robust associations with vascular structural phenotypes therefore presents a window of opportunity. If children following an unhealthy diet trajectory at age 11-12 years shifted to a healthy diet trajectory, this could potentially avert later structural vascular damage.

Conclusion: In this population-derived cohort, following a suboptimal diet trajectory through childhood was associated by age 12 years with higher resting heart rate, lower carotid artery elasticity/distensibility and poorer metabolic health, but not micro- or macro-vascular changes. Because adverse functional arterial changes (such as reduced elasticity) are reversible, especially at younger ages,[56-58] dietary intervention at least up to adolescence could reduce long term CVD risk.

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Supplementary information is available at the International Journal of Obesity's website.

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Figure Legends

Figure 1: Diet trajectories for LSAC's B-Cohort, age 2-11 years. Reprinted with permission from Cambridge University Press (originally printed as Figure 2a[22]). Proportions of children in each trajectory differ from previous publication because we use only the CheckPoint subsample of the full LSAC B-Cohort. Key: dotted line = 95% confidence interval.

Figure 2: Standardised mean differences (i.e. effect size) for preclinical cardiovascular functional phenotypes (from Table 2) by diet trajectory, compared to reference group 'Always Healthy' (dotted line). *linear trend $p<.05$