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Validation of fatty liver disease scoring systems for ultrasound diagnosed non-alcoholic fatty liver disease in adolescents

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Prospective association between dietary fats and fatty liver in a longitudinal cohort population: an 8-year follow-up study from adolescence to young adulthood

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FW was responsible for the study design, statistical analyses, interpreted the results and wrote the manuscript; FP, OTA, LAA, TAM and JKO advised on the statistical analysis. OTA, LAA, TAM, LJB, TAM and WHO contributed to study design, data collection, interpretation of data and revision of the manuscript. All authors approved the final version of the manuscript.

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Acronyms list：

AIC	akaike's information criterion
ALT	alanine transaminase
AST	aspartate aminotransferase
BMI	body mass index
CI	confidence interval
DHA	docosahexaenoic acid
DPA	docosapentaenoic Acid
EPA	eicosapentaenoic acid
FFQ	food frequency questionnaire
FLD	fatty liver disease
FLI	fatty liver index
GBTM	group-based trajectory modelling
GGT	gamma-glutamyl transferase
HDL-C	high-density lipoprotein cholesterol
IQRs	interquartile ranges
LDL-C	low-density lipoprotein cholesterol
MUFA	monounsaturated fats
n-3	omega-3 fatty acids
n-6	omega-6 fatty acids
N6: N3	ratio of omega-6 and omega-3
PUFA	polyunsaturated fats
RCT	randomized controlled trial
RRs	relative risks
SD	standard deviation;

SFA	saturated fats
TFA	total fats
TG	triglycerides
LC-PUFA	long-chain polyunsaturated fatty acids
WC	waist circumference

SUMMARY

Background and Aim

Dietary fat intake has long been associated with fatty liver. Our study aimed to determine the effect of dietary fats on longitudinal fatty liver index (FLI) trajectories from adolescence to young adulthood.

Methods:

Participants in the Raine Study had cross-sectional assessments at ages 14, 17, 20 and 22 years, during which anthropometric measurements and blood tests were obtained. FLI trajectories were derived from the longitudinal FLI results. Dietary fat intake was measured with a semi-quantitative food frequency questionnaire at 14 years and fatty acid intake and composition was estimated.

Results:

Three FLI trajectories were identified and labelled as stable-low (79.1%, N=782), low-high (13.9%, N=132), and stable-high (7%, N=71). Long-chain polyunsaturated fatty acids in the low to high group relative to the stable low group had a relative risk of 1.27 (95% CI 1.10-1.48). Compared to the stable low group, omega-6 and the ratio of omega-6 to omega-3 in the stable high group were associated with an increased relative risk of 1.34 (95% CI 1.02-1.76) and 1.10 (95% CI 1.03-1.16) respectively.

Conclusion:

Our study found that adolescence to young adulthood is an important period in the progression of fatty liver. Dietary fat intake in early adolescence is associated with subsequent fatty liver. High omega-6 fatty acid intake and a high ratio of omega-6 to

omega-3 appear to be associated with fatty liver risk. Diets comprising fewer foods high in omega-6 or with a low N6: N3 fatty acid ratio may reduce the risk of fatty liver.

Introduction

The contribution of an unhealthy diet, including excessive fatty food, to development of fatty liver has been described over nearly two centuries [1]. Non-alcoholic fatty liver disease (NAFLD) is the most common form of fatty liver disease (FLD), with an estimated global prevalence of up to 24% [2]and causes a substantial economic burden to society [3]. The influence of rising obesity prevalence [4,5], sedentary lifestyle [6], and unhealthy dietary patterns [7] on development of fatty liver has previously been reported in adolescents and young adults. A Western dietary pattern incorporating a high dietary fat intake is associated with NAFLD in adolescents [10]. Furthermore, dietary fats are considered to affect the pathogenesis of fatty liver [8].

Although some observational studies [9,10] and dietary intervention studies [11-13] have attempted to clarify the cross-sectional relationship between dietary fats and fatty liver, there is no longitudinal study investigating the relationship between dietary fat intake and fatty liver occurrence and development in adolescents and young adult populations. In the Raine Study, a prospective association between the high-fat "Western dietary pattern" and ultrasound detected NAFLD in adolescents was found [7]. We sought to test the hypothesis that high intake of dietary fat is prospectively associated with fatty liver in adolescents and young adults.

The primary aim of this study was to examine the association between baseline dietary fatty acids intake during early adolescence and subsequent longitudinal fatty liver trajectories as measured by fatty liver index (FLI) from adolescence to young adulthood. Non-invasive diagnostic models, such as FLI, (based on waist circumference, body mass index (BMI) triglycerides and gamma-glutamyl-transferase) are considered to be accurate and validated method of determining NAFLD in population-based

epidemiological studies [14]. To achieve our primary aim, we identified distinct FLI trajectories from 14 to 22 years in the Raine Study and tested the association of early dietary fats in adolescents with these trajectories.

Methods

Study population

We utilised data from the Raine Study, a longitudinal cohort study that started as a randomised controlled trial to study the effects of frequent and repeated ultrasound scans on pregnancy outcomes. The background and methods of the Raine Study have previously been described [7]. The original cohort of pregnant study participants (Gen1) were recruited between 1989 and 1992, at between 16 and 20 weeks gestation, resulting in 2868 live births. Follow up assessments of the offspring (Gen2) cohort has been conducted approximately every 3 years.. Approximately 70% of the Gen2 participants had remained actively involved in the study at the 22-year follow-up. Clinical, biochemical and questionnaire data were collected from serial assessments during antenatal/perinatal stages, infancy, childhood, adolescence and adulthood [15]. Laboratory examination and physical measurement data from the 14, 17, 20 and 22-year follow-ups of the cohort were used in this study (see Figure 2 for detailed information on participant recruitment). Institutional ethics committee approval was obtained from the University of Western Australia Human Research Ethics Committee. Signed informed parental or primary carer consent during Gen1 pregnancy, Gen2 childhood and adolescence, and subsequently by Gen2 adolescents and young adults were obtained before participation in each assessment.

Dietary fat intake assessment

Dietary fat intake at 14 years was obtained from a validated semi-quantitative food frequency questionnaire (FFQ-14) developed by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Adelaide, Australia, as previously described [16] and evaluated [17]. The parents or primary carer completed the FFQ-14 as being representative of usual dietary and nutrient intake in the previous 12 months [18]. The study nurse checked FFQ-14 responses during the clinical follow-up to clarify responses. All data from the FFQ-14 were verified by CSIRO twice, and Australian food composition data were applied to obtain estimates of usual food and nutrient intakes [19]. These included estimates of macro and micronutrients, including specific dietary fats [18] (TFA, total fatty acid; SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; total omega-3 fatty acids (including a-linolenic acid and long-chain n-3), n-3; total omega-6 fatty acids (including alpha-linolenic acid and linolenic acid), n-6; long chain omega-3 PUFA (Eicosapentaenoic Acid, EPA + Docosapentaenoic Acid, DPA + Docosahexaenoic Acid, DHA), LCPUFA; and the ratio of total n-6 to total n-3 fatty acids, n-6:n-3). The FFQ-14 did not collect dietary fish oil supplement intake.

Dietary misreporting

Dietary misreporting can contribute to measurement error in the analysis of diet-disease relationships [20]. Potential dietary misreporting in the Raine study was determined using the Goldberg method [21] (supplementary text 1), which estimates the cut-offs for plausible reporting based on energy intake relative to basal metabolic rate. These cut-off values classify study participants as under-, plausible- or over-reporters of dietary intake. The method has been used widely to identify misreporting from dietary surveys and studies [22]. Because dietary underreporting may be strongly associated with the risk

of overweight [23], we considered excluding under-reporters. However, excluding under-reporters removes participants at the highest risk, reducing our sample size considerably. We therefore created a categorical variable for misreporting where all participant data were included in the analysis. This categorical variable for misreporting was included as a covariate in our regression models. Similarly, when we summarise participants dietary fat intake at baseline, there is a significant difference in the proportion of dietary misreporting between trajectory groups. Therefore we show the dietary data for plausible reporters only in Table 2, and data of the entire cohort with all categories of dietary reporting included (shown in Supplementary Table 1).

Anthropometric and laboratory measurements

At all years, a trained research assistant weighed and measured participants in light clothing for height and weight using a calibrated stadiometer and electronic chair scales. Body mass index (BMI= weight (kg)/ square of the height (m^2)) was calculated, and subjects were categorised as underweight, normal weight, overweight, and obese using the International Obesity Task Force (IOTF) criteria at 14 years [24,25]. Blood was taken by a phlebotomist at the home of the participants from an antecubital vein after an overnight fast. Laboratory assessments were performed in the PathWest Laboratories, Perth, for serum glucose, insulin, alanine transaminase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), triglycerides (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), ferritin, transferrin saturation, high sensitivity C-reactive protein (hs-CRP), adiponectin, and leptin.

Fatty liver index trajectories

The lack of an accurate, non-invasive, easily accessible and affordable diagnostic test has been an important factor limiting research regarding fatty liver in the general population. The FLI is an algorithm based on WC, BMI, TG and GGT. The index was initially developed to detect fatty liver [26]. It is a reliable tool for fatty liver research, recommended by a group of European Societies for diagnosis of fatty liver in epidemiological studies [14] and has been validated in the Raine Study cohort [27]. The FLI has been shown to have the best calibration performance compared to other prediction models attempting to identify fatty liver disease. This suggests that the FLI has a good ability to predict the risk of fatty liver among study participants at the individual level. We hypothesised that FLI is useful as a continuous variable in repeated measurements of longitudinal data to detect changes in fatty liver risk. To answer our research question, we applied a trajectory model to describe the natural history of fatty liver over eight years in Raine Study participants from adolescence (14 years) to young adulthood (22 years) using the FLI (Figure 1).

Covariate assessment

At each follow-up, children's medical status were reported, and the following variables collected. The primary caregivers of the participants were asked to report their annual family income (in Australian dollars) at the 14-year follow-up (2003-2006) and categorised as: <$ 30,000, $30001-50,000, $50001-78,000, and >$78,000. Also, at the 14-year follow-up, hours of physical activity per week were assessed through the International Physical Activity Questionnaire (IPAQ). Participants reported the time and frequency spent in exercising vigorously during physical education at school and outside school. Screen time, including time spent using a computer, was collected

through a questionnaire with response options from ‘none at all’ to ‘4 hours or more’ per day by participants’ primary caregivers.

Statistical analysis

Continuous descriptive data with normal distributions are presented as means ± standard deviation (SD); non-normally distributed data are reported as medians and interquartile ranges (IQRs).

Trajectories were estimated with group-based trajectory modelling (GBTM) [28]. The trajectories were derived by modelling the FLI as a function over time (participants’ age at each follow-up measurement). Data requirements for participants in the model were limited to having FLI data at the 14-year follow-up and at least one other follow-up (17, 20, or 22 years).

The best-fit model was based on the Bayesian Information Criterion (BIC) and the presence of a minimum of 5% of participants per trajectory to ensure stable estimates [28,29]. After setting the number of trajectories and non-significant quadratic and cubic terms were removed, the best optimal model was estimated by fit indices, pragmatic evaluation, and clinical relevance. Fit indices included BIC and Akaike's information criterion (AIC) with the lower the indices, the better the model fit [28] (supplemental text 3 Table A1). After the model was built, we tested its accuracy with the conditions suggested by Nagin [28]: the average posterior probability of assignment of each group was 0.7 or higher; the odds of correct classification was 5.0 or higher; the proportion of samples allocated to a particular group was close to the proportion estimated by the model, and the 99% confidence interval for the estimated proportion was reasonably narrow (supplemental text 3).

Dietary fat exposures were converted to Z-scores in the log multinominal regression models to obtain appropriate clinical interpretations. Log multinominal regression analyses were conducted to estimate relative risks (RRs) and 95% confidence intervals (CIs) with multiple attributes [30] of three distinct FLI trajectories (stable-high FLD risk SH; low-high FLD risk LH) using the "stable-low FLD risk, SL" trajectory groups as a reference. RR and 95% confidence intervals (CIs) were reported for each FLI trajectory as follows: model 1 adjusted for total energy intake plus dietary misreporting; model 2 adjusted for model 1 plus sex; model 3 adjusted for model 2 plus computer viewing, and family income. We used the standard multivariate model instead of nutrient density for energy adjustment. This is because if nutrient density is used, nutrient intake will be confounded by total energy intake (in the opposite direction) as it is divided by total energy when the disease is also associated with total energy intake. BMI was not in the model because the already adjusted covariates such as total energy intake, dietary misreporting and BMI were highly correlated, and additional adjustment for BMI could not be fitted to the model. All analyses were performed using Stata 15.0 for Windows (Stata Statistical Software: College Station, Tx, USA). P values < 0.05 were regarded as statistically significant.

Results

Our study population was derived from comprised 985 Gen2 adolescents aged 14 years. Participant characteristics at the 14-year follow-up are presented by different FLI trajectory groups in Table 1. The overall characteristics of the cohort showed 52% were male, 89% were of Caucasian maternal race, and 42% of the cohort had an annual family income over $70,000 at baseline. Since FLI has no interaction with sex [27], the entire population with no gender breakdown is included in the trajectory analysis model.

From the 985 eligible participants, three different FLI trajectories were identified (Figure 1) (supplementary text 2) and labelled as “stable low fatty liver risk, SL” (79.1%, N=782, trajectory 1), “low to high fatty liver risk, LH” (13.9%, N=132, trajectory 2), and “stable high fatty liver risk, SH” (7%, N=71, trajectory 3). The rising FLI trajectory indicates an increased risk of fatty liver for the individual in the SH group. The majority of participants (79%, N=782) maintained a SL fatty liver risk from 14 to 22 years, while 7% (N=71) and 14% (N=132) were categorised in SH and LH fatty liver risk groups, respectively. For the demographic characteristics in the trajectory groups at baseline (Table 1), the SH group had the highest proportion of males (57.8%), Caucasian maternal race (93.0%), and annual family income less than $35000 (50.7%). The SH group also had higher serum ALT, GGT, triglycerides, insulin and lower HDL-cholesterol, that may represent increased cardiometabolic risk. Lifestyle characteristics, such as dietary misreporting and BMI, were significantly different between the three trajectory groups. In the SH group, nearly 46% were underreporters as identified from the dietary misreporting analysis. The majority of subjects in the SL and LH groups were categorised as within a healthy weight range, whereas 83% of participants in the SH group were categorised as overweight. Among the laboratory biochemical indicators shown in Table 1, the SH group had the highest mean levels of all the biomarkers other than AST and HDL-C.

The baseline dietary fat intake characteristics are shown in Table 2 for plausible reporters only. Considering the group variations in energy requirements, the percentage of energy intake from specific TFA, SFA and MUFA categories of dietary fats are also reported. Energy intake from the fat categories LCPUFA, n-3 and n-6 are not shown because energy levels of these nutrients were very low. All indicators of fat intake characteristics (including absolute value and percentage of energy) were non-normally

distributed. For most indicators, except for n-3 and LCPUFA, the SH risk group had the highest median intake of all dietary fats and percentage of energy compared to the SL and LH groups. For n-3 and LCPUFA, the LH group had the highest median intake level (total n-3 1.3 g/day and LCPUFA 288 mg/day). Except for SFA, the absolute value of most fat categories (TFA, PUFA and MUFA) showed the same trend as the percentage of energy. However, for SFA, the median absolute value of SFA in the LH group is moderate but accounts for the lowest median energy percentage (absolute value: 41.5 (g/day), energy percentage: 14.4%).

Table 3 shows the results from multinomial analysis of the correlation between baseline dietary fats and FLI trajectories with the SL group as the reference group for each dietary fats exposure. The RR of the multinomial analyses shows LCPUFA, n-6 and N-3: N6 at baseline (14 years) were significantly associated with the FLI trajectories after adjusting for all covariates. LCPUFA in the LH group relative to the SL group has a RR of 1.27 (95% CI 1.10-1.48) in model 3. Compared to the SL group, n-6 and N-3: N6 in the SH group has RRs of 1.34 (95% CI 1.02-1.76) and 1.10 (95% CI 1.03-1.16) respectively in the final model.

Supplementary Table 3 shows that the intake of dietary fats was lower in the SH group compared to that of the LH and SL groups. This may be due to the 46.5% under-reporting in the SH group (Table 1). Nevertheless, this relationship is different in n-3 fatty acids and LC-PUFA with the intake of LH group the highest, while the intake of SH group is lower than that of LH group. Supplementary Table 5 shows the RR values for the three LC-PUFA (DPA, EPA and DHA) incorporated in the same regression model. We found that all three lose their significant association with the LH group, while EPA maintains its original risk association trend with the LH group (RR: model

3 1.20 (0.74-1.95). Supplementary Table 6 indicates the component characteristics of LCPUFA intake (plausible reporters only), with DPA being the main component of LCPUFA intake and the highest median level of LCPUFA intake in the LH group among the three trajectory groups.

Discussion

The primary aim of this study was to investigate the prospective association of dietary fats on FLI trajectories, hence fatty liver risk, longitudinally. We identified three different fatty liver index trajectories from a young population in Western Australia and observed a dietary fat intake relationship with these trajectories. The majority of participants were in the SL group, with a sustained low FLI. The SH group had FLI at a relatively persistent high level, while the LH group changed from low to high risk for FLD during follow up. After controlling for total energy, dietary misreporting, sex, computer viewing and family income, intake of LCPUFA, n-6 and N6: N3 at baseline were significantly associated with prospective and longitudinal FLI trajectories over eight years of follow-up.

Our study provides a unique insight into the relationship of longitudinal patterns of dietary fat intake on the natural history of fatty liver from adolescence to young adulthood. We show that fatty liver development follows specific trajectories over time in young populations. FLI, as a predictor of fatty liver, has been widely used in epidemiological studies as an alternative diagnostic tool for fatty liver. The prevalence of datty liver based on FLI in 17-year-old adolescents in the Raine Study was 11.6%[27] Meta-analyses have shown that the prevalence of FLD or NAFLD was 11.3% in children 10-14 years [31], 17.3% in adolescents 15-19 years [32] and 24% in young adults 18-35 years [33]. Our FLI trajectory data show a similar fatty liver disease trend from

adolescents to young adults. These findings are worthy of highlighting, since the chronicity and severity of fat accumulation in the liver is associated with subsequent risk of type II diabetes and cirrhosis later in life [2]. It also demonstrates the importance of the transition from adolescence to young adulthood as a critical period for health promotion and health interventions to reduce the impacts of chronic liver disease and cardiometabolic risk.

Our main finding is that a diet high in n-6 fatty acids or a high N6: N3 ratio at 14 years of age associates with an increased risk of FLD from adolescence to young adulthood. This implicates n-6 fatty acids as a potential risk factor in the development of FLD. Moreover, these findings are similar to previous studies in which cross-sectional associations between N6: N3 and obesity[34] and fatty liver risk [35] were observed. In a typical Western diet, the ratio of N6: N3 is usually 15-16:1, rather than the recommended 1-4:1, that is considered the healthy range [36]. Nuts and seeds are rich in n-6, which is why large amounts of n-6 can be found in refined vegetable oils such as palm oil and sunflower oil and foods cooked with these vegetable oils. Vegetable oils are a widely used source of fatty acids in the food industry for processed snacks, fast foods, cakes and cured meats that are also rich in n-6 and high in ratio of N6: N3[37]. These findings are biologically plausible because desaturase and elongase tend to metabolise n-3, thereby competing with n-6 and reaching a dynamic equilibrium. Thus, in the presence of large amounts of n-6 PUFA in the diet, its metabolites such as arachidonic acid and its derivatives disrupt the original anti/pro-inflammatory imbalance by increasing thrombosis and inflammation [38,39].

Another interesting finding suggested that LCPUFA, especially EPA, could be a risk factor for fatty liver. In fact, even though EPA and DHA are often grouped together as

omega-3 fatty acids and are thought to be beneficial for lipid metabolism, differences are being reported regarding the mechanisms of EPA versus DHA [40-43]. In a dietary fatty acid study uing mice as subjects [40,44], EPA was found to be associated with elevated hepatic inflammatory markers and poorer performance in reducing liver fat or improving liver metabolism. A more credible human RCT study showed that EPA supplementation was weaker than DHA in lowering serum inflammatory markers and blood lipids [45]. However, so far there are no data from human studies highlighting this difference in hepatic lipid metabolism. A human epidemiological study from a prospective nested case-control cohort found a positive risk association (although not statistically significant) of EPA with the risk of Crohn's disease [46]. This is similar to the findings in our study, where only EPA appeared to exhibit a positive trend (not statistically significant) with increased FLD risk (Supplementary Table 5). The reason for this is unclear, although basic research suggests that generally, EPA and DHA down-regulate the inflammatory response, though perhaps less effectively than DHA [43,45]. There are also studies reporting that EPA can be pro-inflammatory [44]. Otherwise, for the same food source, EPA and DHA should be observed to have an isotropic effect rather than an opposite effect (Supplementary Table 5). Another possible reason is the different food sources of dietary fats. EPA is available from a wider range of food sources, such as edible oils, rather than being limited to oily fish alone. The process of dietary data collection resulted in the association of EPA with potential risk factors thus making a spurious association in the analysis. This suggests that more research should be done on dietary fatty acid sources in this population, and if DHA and EPA dietary sources are different, differences in their utility performance could potentially also be explained.

Our study validates findings in nutritional epidemiology regarding dietary misreporting, which is often associated with BMI status; educational level; and age [47,48] and can be especially vulnerable to bias when we evaluate dietary-metabolic disease associations. Differences in dietary characteristics between plausible (table 2) and implausible (supplementary table 1) populations may result from overweight and obese people under-reporting their intake of dietary fats [49].

Strengths and limitations

To our knowledge, this study is the first to explore the association between dietary fat intake of adolescents with the longitudinal natural development of fatty liver into young adulthood. The strengths of our study include the use of data from a large prospective cohort which can prospectively collect dietary information to minimise recall bias representative of the general adolescent and young adult population, a focus on the natural history of FLD risk from adolescence to young adulthood and use of an objective and validated fatty liver screening tool for longitudinal data analysis over eight years.

Our study has several limitations. First, proper interpretation of the results of the study requires consideration of measurement errors in self-reported dietary data, unavoidable when using an FFQ [50]. We minimised this impact by applying a less biased instrument (3-day food record) and performing an internal calibration of the FFQ data [17] finding relative validity. Dietary misreporting is introduced into the analysis as a covariate factor to achieve better statistical modelling. The energy adjustment method was also applied to minimise the impact of FFQ measurement errors in statistical modelling. Second, our data come from a pregnancy cohort and participants were recruited (Gen 2) predominantly in a relatively concentrated age range, so it is not possible to

determine whether our findings are applicable to other populations. Finally, dietary fatty acid exposure in our study was assessed only at age 14 years. Dietary habits during adolescence may change over time. The use of only one prospective assessment of dietary intake is a limitation of our study. We have also not accounted for degrees of physical activity.

Our study reveals that aspects of dietary fat intake are associated with the future risk of FLD in young populations. While dietary fat consumption at age 14-years is unlikely to independently lead to an increased risk of FLD eight years later, dietary habits established during adolescence are likely to persist into adulthood, including into the reproductive years [51]. Interestingly, we examined changes in the trajectory of dietary patterns from adolescence to early adulthood (from 14, 17 to 20 years of age) in the Raine study Gen2 population[52] and found that 21% of men consuming mainly the Western Dietary Pattern Score had a stable, significant growth trajectory over time suggesting that dietary patterns established during adolescence are likely to persist into early adulthood, especially among males. This study supports the idea that specific populations can still benefit from early dietary interventions. Therefore, family diet education or intervention may be a viable way to prevent or reduce the rising prevalence of fatty liver among younger populations.

Future nutritional epidemiological studies should focus on the pathways by which n-6 fatty acids are associated with FLD, such as the mediation of inflammatory factors. Additionally, a focus on risk factors for the development of FLD in potential risk populations is recommended, such as validating the relationships between EPA and metabolism-related outcomes in larger populations.

Conclusions

Our study shows that some aspects of fatty acid intake in early adolescence are associated with FLD trajectories from adolescence to young adulthood, highlighting a critical period for forecasting the development or perpetuation of fatty liver. Diets to improve fatty acid balance in the diet to protect against FLD need to be promoted that include a low N6: N3 fatty acid diet and fewer foods high in n-6.

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Table 1: Characteristics of study population at baseline (14 years) by trajectory groups from 14 to 22 years in the Raine Study

Characteristic	SL group	LH group	SH group	Total
N	782	132	71	985
General				
Sex † (proportion of males) (%)	50.1	56.8	57.8	51.6
Maternal race ‡	87.6	91.7	93.0	88.5
(proportion with Caucasian mother) (%)				
Annual family income	N=770	N=131	N=70	N=971
at baseline (%) **				
<35000	19.5	23.7	50.7	22.4
35000-70000	36.8	35.9	27.9	35.9
>70000	43.8	40.5	21.4	41.7
Child lifestyle at 14 years				
Dietary Misreporting (%)**				
Underreporting	22.3	29.6	46.5	25.0
Plausible reporting	66.2	62.1	52.1	64.7
Overreporting	11.5	8.3	1.4	10.3
BMI (%)**				
Underweight	3.1	0	0	2.4
Healthy weight	83.6	40.0	2.8	72.0
Overweight	11.8	3.8	14.1	16.0
Obese	1.5	18.2	83.1	9.6
Physical activity (%)	N=628	N=102	N=51	N=781

once month or less	9.7	9.8	9.8	9.7
1-3 times per week	57.2	53.9	66.7	57.4
4+ times per week	33.1	36.3	23.5	32.9
Computer viewing (%)	N=778	N=135	N=70	N=983
< 2 h per day	80.2	71.2	75.7	78.5
2–4 h per da	13.5	18.2	17.2	14.5
> 4 h per day	6.3	10.6	7.1	7.0
Biochemistry (mean and standard deviation)				
ALT (U/L) **	16.19(6.77)	17.74(10.38)	23.06(10.35)	16.89(7.84)
AST (U/L) **	46.58(2.51)	46.35(2.67)	45.90(2.67)	46.50(2.52)
GGT (U/L) **	11.10(4.31)	11.77(3.72)	16.55(6.75)	11.58(4.67)
Total cholesterol (mmol/L)	4.17(0.68)	4.09(0.76)	4.30(0.81)	4.17(0.70)
Triglycerides (mmol/L) **	0.95(0.43)	1.08(0.45)	1.51(1.18)	1.01(0.54)
Insulin (mU/L) **	11.16(7.40)	13.01(6.27)	22.91(14.13)	12.25(8.49)
HDL-C (mmol/L) **	1.44(0.32)	1.29(0.28)	1.13(0.01)	1.39(0.32)
LDL-C (mmol/L)	2.30(0.61)	2.31(0.66)	2.47(0.71)	2.31(0.63)

Footnote:

ALT: alanine transaminase; AST: aspartate aminotransferase; BMI: body mass index; FFQ: food frequency questionnaire; GGT: gamma-glutamyl transferase; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; LH: low risk to high risk; SH: stable high risk, SL: stable low risk;

The results are presented as means and standard deviations except for percentages.

* for P<0.05 and ** for P<0.01 for the difference within groups.

†； baby's sex was recorded at birth.

‡: Maternal race data were collected at study recruitment 16-20 weeks gestation

Table 2 FFQ dietary fat intake characteristics of participants at age 14 years presented as median and interquartile ranges (Q1, Q3) (Plausible reporters only) in the Raine Study

Dietary fats at 14 years (g/day)	SL group	LH group	SH group	P-value
N	518	82	37	
TFA	89.9(75.9,104.6)	98.3(79.3,113.8)	102.1(88.0,117.3)	0.65
energy from TFA (%)	34.9(31.5,38.6)	35.2 (31.7,39.0)	36.3(32.1,39.3)	0.75
SFA	39.2(32.3,47.2)	41.5(32.7,49.4)	44.6(38.1,53.3)	0.77
energy from SFA (%)	15.3(13.1,17.4)	14.4(12.3,16.9)	15.5(14.2,17.7)	0.15
PUFA	12.6(9.7,17.7)	16.1 (11.5,20.2)	16.8(10.7,20.3)	0.10
energy from PUFA (%)	5.1(3.8,6.3)	5.7(4.0,7.1)	5.8(3.9,7.0)	0.12
MUFA	31.2(26.1,36.1)	34.8(27.4,42.3)	34.8(30.5,40.0)	0.55
energy from MUFA (%)	12.1(10.7,13.3)	12.3(10.6,14.1)	12.4(11.1,13.4)	0.32
n-3	1.2(1.0,1.5)	1.3(1.0,1.8)	1.2(1.1,1.5)	**0.05**
LCPUFA (mg/day)	231.0(164.8,306.9)	288.4(193.3,414.9)	237.0(167.2,301.1)	**0.01**
n-6	10.3(7.8,14.6)	12.9(8.8,17.2)	14.5(9.2,17.2)	0.08
N6: N3	9.1(6.6,11.9)	9.7(6.8,12.5)	12.0(7.1,13.5)	**0.01**

Footnote:

DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; LCPUFA: long-chain fatty acids (EPA + DPA + DHA); LH: low risk to high risk; MUFA: monounsaturated fats; n-3: omega-3 fatty acids; N6: N3: the ratio of total n-6 and total n-3 fatty acids; n-6: omega-6 fatty acids; PUFA: polyunsaturated fats; SFA: saturated fats; SH: stable high risk; SL: stable low risk; TFA: total fats;

The results are presented as median and interquartile ranges (Q1, Q3).

Table 3. Log multinomial regression models for each dietary fat (Z-score) at 14 years and different fatty liver index trajectories in the Raine Study (stable low risk as the reference group).

Dietary fats (z-score) at 14 years	Low to high (LH) risk group	P-value	Stable high (SH) risk group	P-value
Total fat z-score				
Model 1 (N=985)	0.94(0.64-1.37)	0.764	1.43(0.80-2.55)	0.225
Model 2 (N=985)	0.98(0.68-1.43)	0.920	1.58(0.90-2.78)	0.110
Model 3 (N=966)	1.01(0.70-1.46)	0.949	1.55(0.90-2.65)	0.112
Saturated fat z-score				
Model 1	0.80(0.61-1.04)	0.100	1.10(0.74-1.63)	0.630
Model 2	0.82(0.63-1.08)	0.158	1.19(0.81-1.75)	0.370
Model 3	0.84(0.65-1.11)	0.225	1.17(0.83-1.66)	0.361
Polyunsaturated fat z-score				
Model 1	1.14(0.94-1.37)	0.186	1.26(0.95-1.65)	0.107
Model 2	1.14(0.94-1.38)	0.191	1.28(0.96-1.71)	0.090
Model 3	1.15(0.95-1.39)	0.164	1.20(0.89-1.61)	0.228
Monounsaturated fats z-score				
Model 1	1.15(0.81-1.61)	0.438	1.44(0.85-2.42)	0.173
Model 2	1.15(0.82-1.60)	0.424	1.43(0.88-2.33)	0.153
Model 3	1.16(0.84-1.62)	0.365	1.56(0.96-2.54)	0.074
Total omega-3 z-score				
Model 1	1.14(0.97-1.33)	0.119	0.86(0.61-1.19)	0.362
Model 2	1.14(0.97-1.33)	0.112	0.89(0.64-1.23)	0.482

Model 3	1.13(0.97-1.32)	0.126	0.99(0.71-1.38)	0.942
long-chain fatty acids				
(EPA + DPA +DHA) z-score				
Model 1	**1.28(1.11-1.47)**	**<0.001**	0.96(0.73-1.25)	0.744
Model 2	**1.28(1.11-1.47)**	**0.001**	0.91(0.70-1.18)	0.485
Model 3	**1.27(1.10-1.48)**	**0.001**	0.96(0.75-1.24)	0.782
Total omega-6 z-score				
Model 1	1.10(0.91-1.32)	0.319	**1.33(1.05-1.70)**	**0.019**
Model 2	1.10(0.92-1.33)	0.297	**1.38(1.08-1.78)**	**0.010**
Model 3	1.12(0.93-1.34)	0.240	**1.34(1.02-1.76)**	**0.035**
Total n-6: total n:3 z-score				
Model 1	0.99(0.94-1.03)	0.593	**1.11(1.05-1.17)**	**<0.001**
Model 2	0.99(0.95-1.04)	0.641	**1.12(1.06-1.19)**	**<0.001**
Model 3	0.99(0.95-1.04)	0.709	**1.10(1.03-1.16)**	**0.003**

Footnote:

DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; LH: low risk to high risk; SH: stable high risk; SL: stable low risk.

The results are presented as means and standard deviations.

Model 1 adjusted for total energy+ misreporting

Model 2 adjusted for 1+ sex

Model 3 adjusted for 2+ computer viewing + family income.