A healthy lifestyle during adolescence was inversely associated with fatty liver indices in early adulthood: findings from the DONALD cohort study

Maike Elena Schnermann\textsuperscript{1}, Christina-Alexandra Schulz\textsuperscript{1}, Ines Perrar\textsuperscript{1}, Christian Herder\textsuperscript{2,3,4}, Michael Roden\textsuperscript{2,3,4}, Ute Alexy\textsuperscript{1} and Ute Nöthlings\textsuperscript{1*}

\textsuperscript{1}Institute of Nutrition and Food Sciences, Nutritional Epidemiology, University of Bonn, Bonn 53115, Germany
\textsuperscript{2}Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research, Heinrich Heine University Düsseldorf, Düsseldorf, Germany
\textsuperscript{3}German Center for Diabetes Research (DZD), Partner Düsseldorf, München-Neuherberg, Germany
\textsuperscript{4}Department of Endocrinology and Diabetology, Medical Faculty, University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

(Submitted 4 February 2022 – Final revision received 29 March 2022 – Accepted 19 April 2022)

Abstract
A healthy lifestyle during adolescence is associated with insulin sensitivity or liver enzyme levels and thus might contribute to the prevention of non-alcoholic fatty liver disease (NAFLD). Therefore, we examined the association between adherence to a hypothesis-based lifestyle score including dietary intake, physical activity, sedentary behaviour, sleep duration and BMI in adolescence and fatty liver indices in early adulthood. Overall, 240 participants of the DOrtmund Nutritional and Anthropometric Longitudinally Designed study completed repeated measurements of lifestyle score factors during adolescence (females: 8.5–15.5 years, males: 9.5–16.5 years). Multivariable linear regression models were used to investigate the association between adolescent lifestyle scores and NAFLD risk (hepatic steatosis index (HSI) and fatty liver index (FLI)) in early adulthood (18–30 years). Participants visited the study centre 4–9 times during adolescence and achieved on average 2.8 (min: 0.6, max: 5) out of five lifestyle score points. Inverse associations were observed between the lifestyle score and fatty liver indices (HSI: \(\beta = -3.9, P < 0.0001\), FLI: \(\beta = -32.4\%\), (95\% CI \(-42.9, -20.4\)), \(P < 0.0001\)) in the overall study population. Sex-stratified analysis confirmed these results in men, while inverse but non-significant associations were observed in women \((P > 0.05)\). A higher lifestyle score was associated with lower HSI and FLI values, suggesting that a healthy lifestyle during adolescence might contribute to NAFLD prevention, predominantly in men.

Our findings on repeatedly measured lifestyle scores in adolescents and their association with NAFLD risk in early adulthood warrant confirmation in larger study populations.

Key words: Lifestyle: Lifestyle score; Adolescence: Fatty liver index; Hepatic steatosis index; Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is the leading cause of liver disease worldwide\textsuperscript{(1)}, which in recent years increasingly occurred in young people\textsuperscript{(2)}. In addition, NAFLD has been shown to be an important risk factor for later-life chronic diseases, such as type 2 diabetes\textsuperscript{(3)}, cardiovascular disease\textsuperscript{(4)} or cancer\textsuperscript{(5,6)}. Western dietary patterns high in sugar and saturated fat\textsuperscript{(7)}, physical inactivity\textsuperscript{(8)} and elevated body weight\textsuperscript{(9)} are discussed as risk factors for the development of NAFLD. Therefore, prevention of NAFLD through a healthy lifestyle across the lifespan may be possible.

Precursors of the later manifestation of NAFLD, such as obesity, reduced insulin sensitivity, hepatic fat accumulation or chronic inflammation can already occur earlier during life, for example, in childhood or adolescence\textsuperscript{(10,11)}. The period of adolescence and puberty is subject to many changes in lifestyle and metabolism, including hormonal changes\textsuperscript{(12,13)}. In addition, since many habits adopted in adolescence persist into adulthood\textsuperscript{(14)}, this period also represents a window of opportunity for the establishment of healthy lifestyle behaviours.

A lifestyle score can be created to combine a number of lifestyle factors in one variable with higher scorings relating to more favourable behaviour. Indeed, several studies showed inverse associations between a healthy lifestyle score and NAFLD risk in adult populations\textsuperscript{(15–17)}.

In addition, some studies have investigated single lifestyle factors and indicators of NAFLD in adolescents such as insulin resistance\textsuperscript{(18)} or increased liver enzyme activities\textsuperscript{(19)}. Results from cross-sectional studies showed that Western dietary patterns

Abbreviations: DONALD, DOrtmund Nutritional and Anthropometric Longitudinally Designed; FLI, fatty liver index; HSI, hepatic steatosis index; MVPA, moderate-to-vigorous physical activity; NAFLD, non-alcoholic fatty liver disease.

* Corresponding author: Dr U. Nöthlings, email noethlings@uni-bonn.de
were associated with the prevalence of NAFLD (20), insulin resistance (21), liver fat content (22) or even risk of type 2 diabetes in young adulthood (23). Conversely, a healthy diet, rich in fruits, vegetables and whole grains and limited in the intake of sugar-sweetened beverages and red meat, was inversely associated with NAFLD risk (24).

In addition, a number of epidemiological studies supported the hypothesis that physical activity was inversely associated with liver enzyme levels (25, 26) or a lower liver fat content (25). In a recent systematic review and meta-analysis of intervention studies, physical activity was associated with reduced liver fat content and liver enzyme values (27). The role of sedentary behaviour is less clear; results of cross-sectional studies suggested that sedentary behaviour was negatively associated with insulin sensitivity (28) and positively with insulin resistance (29), whereas inconsistent associations were found with liver enzyme levels (26, 30). In addition, obesity between 6 and 18 years of age was strongly associated with NAFLD risk in adulthood (31). Furthermore, recent research proposed a link between insulin resistance and sleep duration in adolescence. In a systematic review, inconsistent directions of an association with sleep were found, but inverse associations of short sleep duration with insulin resistance and sensitivity predominated (32).

The aim of the present study was (1) to calculate a lifestyle score for behaviour in adolescence including the factors healthful diet, physical activity, sedentary behaviour, sleep duration and BMI and (2) to analyse its prospective association with fatty liver indices in young adulthood.

Research design and methods

Study design

For the present analysis, we used data from the DOrtmund Nutritional and Anthropometric Longitudinally Designed (DONALD) cohort study. DONALD is an ongoing open cohort study, which was initiated in 1985 in Dortmund, Germany. Details on recruitment, study design and methods have been published previously (33). In brief, information about dietary intake, anthropometric measurements and lifestyle questionnaires was collected annually from all study participants between infancy and young adulthood. In addition, fasting blood samples for each participant were collected from age 18 onwards. The DONALD study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the University of Bonn (ethics numbers: 098/06 and 185/20). All examinations were conducted with written informed consent from study participants themselves (≥ 16 years) or their parents.

Study population

Participants of the DONALD study, who provided at least one measurement of all five lifestyle factors (diet, physical activity, sedentary behaviour, sleep duration and BMI) at the same age during adolescence (male: between 9.5 and 16.5 years of age; female: between 8.5 and 15.5 years of age), were included (n = 32). Of these, 256 participants additionally provided a fasting blood sample and anthropometric measurements to calculate fatty liver indices. Further, we excluded participants who were born pre-term (<36 gestation week) or post-term (>42 gestation week) (n = 7), part of multiples (n = 6) or had a birth weight <2500 g (n = 3). The final study population comprised in total 240 participants (Fig. 1). Online Supplementary Fig. S1 shows additionally a diagram depicting the repeated assessment of the lifestyle factors during adolescence (exposure) and the one-time assessment of anthropometric and blood markers for the calculation of fatty liver indices in young adulthood (outcome).
Table 1. Lifestyle factors and scoring system of the developed lifestyle score

<table>
<thead>
<tr>
<th>Lifestyle factor and scoring criteria</th>
<th>Points</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall score</td>
<td>0–5</td>
<td>DGE(38), USDA(39), WHO(40)</td>
</tr>
<tr>
<td>Dietary intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consumption of &lt;3 food groups/d</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Consumption of ≥3 food groups/d</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>MVPA</td>
<td></td>
<td>WHO(41)</td>
</tr>
<tr>
<td>&lt;60 min/d</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>≥60 min/d</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sedentary behaviour</td>
<td></td>
<td>Graf et al.(42)</td>
</tr>
<tr>
<td>8–5–11 years: &gt;60 min/d</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>12–16 years: &gt;120 min/d</td>
<td>1</td>
<td>AASM(43)</td>
</tr>
<tr>
<td>Sleep duration</td>
<td></td>
<td>Kromeyer-Hauschild et al.(38)</td>
</tr>
<tr>
<td>8–5–12 years: &lt;9 h/d</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>≥12 h/d</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>13–16 years: ≥9 h/d</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>13–16 years: ≥10 h/d</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>BMI SDS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight or underweight</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Normal weight</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

DGE, German Nutrition Society; USDA, United States Department of Agriculture; MVPA, moderate-to-vigorous physical activity; AASM, American Academy of Sleep Medicine; SDS, standard deviation score.

* Recommended servings were considered for the food groups fruits, vegetables, whole grains, sugar-sweetened beverages, fish and red meat/SAUSAGES.

Repeated assessment of lifestyle factors during adolescence

At each annual assessment, information on dietary intake data was collected via 3-d weighed dietary records on consecutive days. The procedure for collecting and handling dietary data has been described in detail previously(33). The in-house database LEBSTAB(34) was used to calculate intake in terms of food groups (fruits, vegetables, whole grains, sugar-sweetened beverages, fish and red meat) in g/d. A standardised questionnaire based on the validated Adolescent Physical Activity Recall Questionnaire(35) was used to collect daily moderate-to-vigorous physical activity (MVPA) and sedentary behaviour (while watching TV or engaging in school homework) on weekdays and weekends. An interview-based questionnaire was used to assess sleep duration per day. Trained nurses assessed weight and height with the participants dressed in undergarments only and barefoot for the calculation of BMI (kg/m²). BMI SDS during adolescence was calculated using German age- and sex-specific BMI percentiles(36).

Adolescent lifestyle score (exposure)

We previously developed an adolescent lifestyle score(37) including the following factors: dietary intake, MVPA, sedentary behaviour, sleep duration and BMI SDS. For each lifestyle factor, one point was given when fulfilling the underlying reference, for example a recommendation (Table 1). Healthy behaviours were defined as follows: (1) consumption of recommended servings per day in ≥3 food groups(38–40), (2) ≥60 min of MVPA per day(41), (3) being within the recommended range of hours per day of sedentary behaviour(42), (4) being within the recommended range of hours of sleep per day(43) and (5) being normal weight(36). Points were summed to a lifestyle score ranging from 0 to 5 points. To describe the lifestyle during adolescence which in our study covered between 1 and 8 measurements per participant, the arithmetic mean of all available lifestyle scores was calculated.

Fatty liver indices (outcome)

Blood withdrawal and anthropometric measurements were performed in young adults (≥18 years). Venous blood samples were centrifuged at 4°C within 15 min after withdrawal and stored at −80°C at the study centre. Liver enzymes (alanine-aminotransferase, aspartate-aminotransferase and γ-glutamyltransferase) and plasma triglycerides (TG) were measured using a Roche/Hitachi Cobas c311 analyser (Roche Diagnostics) at the German Diabetes Center (DDZ) as described(44). Fasting plasma glucose was determined on a Roche/Hitachi Cobas c511 analyser. Body weight, height and waist circumference were assessed by trained nurses. BMI (kg/m²) was calculated as described above.

To determine NAFLD, we used the hepatic steatosis index (HSI) and the fatty liver index (FLI). These two indices were calculated as follows:

\[
\text{HSI} = 8 \times (\text{alanine-aminotransferase/}\text{aspartate-aminotransferase}) + \text{BMI} \times (\text{if female}, +2 \text{if diabetes mellitus})
\]

\[
\text{FLI} = e^{x(1 + e^{x})} \times 100
\]

with \(x = 0.953 \times \ln(\text{TG}) + 0.139 \times \text{BMI} + 0.718 \times \ln(\gamma\text{-glutamyltransferase}) + 0.053 \times \text{waist circumference} - 15.745\).

Diabetes was defined according to the American Diabetes Association with a fasting plasma glucose ≥126 mg/dL(47).

Assessment of covariates

Family and socio-economic characteristics (parental education, employment and smoking in the household) were collected via interviews at regular intervals parallel to the measurements of the predictors during adolescence. Maternal height and weight were measured as described above. Maternal overweight was classified as BMI >25 kg/m². Gestational and birth parameters (pregnancy duration (weeks), maternal weight gain during pregnancy (kg), birth height (cm) and weight (g)) were collected from the German standardised pregnancy document (‘Mutterpass’). Exclusive breast-feeding duration (weeks) was recorded via repeated parental interviews during the first year of life. Additionally, age at outcome assessment and time difference between mean age in adolescence and fatty liver indices assessment were considered as potential confounder.

Statistical analysis

Participants’ characteristics across categories of the adolescent lifestyle score (<2, 2–2.9, 3–3.9 and ≥4 points) were presented as median (25th percentile; 75th percentile) for continuous variables and as relative frequencies (%) for categorical variables unless otherwise noted. Trends across categories of the adolescent lifestyle score were tested using age- and sex-adjusted linear
regression models. Results from the regression analyses are presented as adjusted β-estimate (95% CI).

The longitudinal association between the adolescent lifestyle score and fatty liver indices in young adulthood was analysed using multivariable linear regression models with the lifestyle score as continuous variable. Both outcome variables, HSI and FLI, were log-transformed to achieve normal distribution. For the interpretation, log-transformed outcome variables were back transformed [(e^β-estimate – 1) × 100] to describe the percentage change of the dependent variable. Formal interaction analyses indicated sex interactions between the adolescent lifestyle score and sex for the FLI (Pinteraction = 0.03), but not for the HSI (Pinteraction = 0.06). In addition to analysing the overall sample, we therefore stratified our analysis by sex.

The basic model was adjusted for age at outcome assessment and sex (for overall sample analyses only). Potential confounders were included in the multivariable adjusted model if they significantly modified the predictor–outcome association (change in β-estimate ≥10%)⁴⁹. Covariates considered were (1) early life factors (pregnancy duration, mother’s weight gain during pregnancy, birth size, birth weight and full breast-feeding duration (≥4 month)), (2) parental socio-economic characteristics (education, employment, smoking in the household and maternal overweight) and (3) time difference between mean age in adolescence and fatty liver indices assessment. Finally, the multivariable adjusted model was additionally adjusted for maternal overweight (BMI > 25 kg/m²).

Further analyses were conducted to test the robustness of our findings. First, as existing evidence confirmed waist-to-height ratio as a suitable predictor of NAFLD⁴⁹⁵⁰, we replaced the lifestyle score factor BMI SDS with waist-to-height ratio (cut-off = 0.469⁵⁰). Second, to understand the impact of each lifestyle factor, we calculated five modified lifestyle scores based on only four lifestyle factors instead of five, omitting one factor at a time. The respective multivariable model was additionally adjusted for the omitted lifestyle factor. Third, we investigated the association between increasing adherence to lifestyle recommendations compared with the lowest lifestyle score category (≤2 points).

In addition, we performed sensitivity analyses in subpopulations of (1) participants who provided data on any lifestyle factor at least twice during adolescence (n 221) (as opposed to at least once in the main analyses) and (2) participants with most reliable dietary data, that is, excluding those at risk of potential underreporting (n 217). For potential underreporting of energy intake, dietary intake data were tested according to the Goldberg approach⁵¹, using paediatric cut-offs for the relationship between reported total energy intake and estimated basal metabolic rate⁵². Power calculations based on partial correlation coefficients showed that the considered study population was large enough to detect associations between the lifestyle score and fatty liver indices with a power of > 98%. All statistical analyses were conducted using SAS (Version 9.4). Statistical significance was defined as a P-value < 0.05.

Results

Table 2 summarises main characteristics of study participants during adolescence. Per participant, between one (n 19, 7.9%) and eight (n 1, 0.4%) lifestyle score assessments (median: 6, 25th percentile; 3, 75th percentile: 7) were available. Overall, the participants achieved a mean adolescent lifestyle score of 2.8 with a minimum of 0 points and a maximum of 5 points. The majority of the study participants achieved between 2 and 4 points (82%), whereas only 5% of the participants achieved a lifestyle score ≥4 points. Across increasing categories of the lifestyle score, the proportion of participants with maternal overweight decreased (Ptrend = 0.0140). With regard to the single lifestyle factors, the highest category (≥4 points) often showed the most desirable lifestyle behaviour, for example, fruit and vegetable consumption increased with increasing lifestyle score, but not in a strictly monotonic manner (Ptrend < 0.0001 and Ptrend = 0.0191, respectively). In addition, MVPA increased almost threefold between categories (Ptrend < 0.0001). Sleep duration and BMI SDS decreased between the categories of the adolescent lifestyle score (Ptrend < 0.0001 and Ptrend = 0.0001, respectively). Participants’ reference fulfilment across categories of the lifestyle score showed the lowest fulfilment for sedentary behaviour, ranging from 0 to 53.9% (Ptrend < 0.0001) and the highest fulfilment for having normal weight (16.7–92.5%, Ptrend < 0.0001) (online Supplementary Table S1). With increasing categories of the adolescent lifestyle score, except for the highest category, participants had a more favourable risk marker profile in early adulthood (Table 3).

Table 4 shows the percentage change (95% CI) of the fatty liver indices per 1-point increase in the adolescent lifestyle score. After adjustments of the multivariable adjusted model, an increase in the adolescent lifestyle score by 1 point was associated with a reduction in both fatty liver indices, HSI and FLI, in the overall sample (–5.8% (95% CI (–8.3, –3.1), P < 0.0001) and –32.4% (95% CI (–42.9, –20.0), P < 0.0001), respectively). Sex-stratified analyses showed similar associations among men (HSI: –7.8% (95% CI (–11.5, –4.0), P = 0.0001) and FLI: –42.0% (95% CI (–54.2, –26.7), P < 0.0001), whereas no associations were found for women (P > 0.05). When replacing the factor BMI in the lifestyle score with waist-to-height ratio as a suitable predictor of NAFLD, results remained unchanged (online Supplementary Table S2). In the overall sample, modified lifestyle scores without either diet, MVPA, sedentary behaviour, sleep duration or BMI SDS also showed inverse associations with the fatty liver indices (online supplementary S3 Table). No difference was observed between the highest lifestyle score category (≥4 points) in the overall sample (online Supplementary Table S4).

Discussion

A healthy lifestyle during adolescence, including the factors diet, MVPA, sedentary behaviour, sleep duration and BMI SDS, was inversely associated with fatty liver indices in early adulthood. Findings were significant for men and non-significant for women, showing a preventive potential predominantly in men.
Table 2. Characteristics in adolescence across categories of the adolescent lifestyle score*  
(Median and percentiles; mean values and minimum–maximum values)

<table>
<thead>
<tr>
<th>Lifestyle score categories (points)</th>
<th>&lt;2</th>
<th>2–2.9</th>
<th>3–3.9</th>
<th>≥4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>25th, 75th percentile</td>
<td>Median</td>
<td>25th, 75th percentile</td>
</tr>
<tr>
<td>Basic characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>30</td>
<td>101</td>
<td>96</td>
<td>13</td>
</tr>
<tr>
<td>Adolescent lifestyle score‡</td>
<td>1.5</td>
<td>2.4</td>
<td>3.3</td>
<td>4.2</td>
</tr>
<tr>
<td>Mean age at lifestyle assessment (years)</td>
<td>13.0</td>
<td>13.0, 14.5</td>
<td>13.0</td>
<td>12.2, 14.0</td>
</tr>
<tr>
<td>Male participants§</td>
<td>60.0</td>
<td>45.5</td>
<td>53.1</td>
<td>69.2</td>
</tr>
<tr>
<td>Total energy intake (kcal/d)</td>
<td>1882</td>
<td>1676, 2291</td>
<td>1857</td>
<td>1648, 2083</td>
</tr>
<tr>
<td>Maternal overweight (BMI &gt;25 kg/m²)</td>
<td>66.7</td>
<td>35.6</td>
<td>42.7</td>
<td>7.7</td>
</tr>
<tr>
<td>Parental education (&gt;12 years)</td>
<td>76.7</td>
<td>72.3</td>
<td>71.9</td>
<td>84.6</td>
</tr>
<tr>
<td>≥2 lifestyle assessments‡</td>
<td>96.7</td>
<td>93.1</td>
<td>92.7</td>
<td>69.2</td>
</tr>
<tr>
<td>Lifestyle factors during adolescence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruits (serving/d)</td>
<td>1.4</td>
<td>1.1, 2.0</td>
<td>1.5</td>
<td>1.1, 1.9</td>
</tr>
<tr>
<td>Vegetables (serving/d)</td>
<td>1.6</td>
<td>0.9, 2.4</td>
<td>1.5</td>
<td>0.9, 2.1</td>
</tr>
<tr>
<td>Whole grain (serving/d)</td>
<td>0.6</td>
<td>0.3, 0.9</td>
<td>0.5</td>
<td>0.2, 0.9</td>
</tr>
<tr>
<td>Sugar-sweetened beverages (serving/d)</td>
<td>1.1</td>
<td>0.2, 1.8</td>
<td>0.8</td>
<td>0.4, 1.7</td>
</tr>
<tr>
<td>Fish (serving/d)</td>
<td>0.2</td>
<td>0.1, 0.3</td>
<td>0.1</td>
<td>0.1, 0.2</td>
</tr>
<tr>
<td>Red meat and sausages (serving/d)</td>
<td>1.7</td>
<td>1.4, 2.5</td>
<td>1.9</td>
<td>1.4, 2.6</td>
</tr>
<tr>
<td>MVPA (min/d)</td>
<td>38.7</td>
<td>23.2, 56.6</td>
<td>61.0</td>
<td>42.9, 80.5</td>
</tr>
<tr>
<td>Sedentary behaviour (min/d)</td>
<td>188.9</td>
<td>162.9, 221.0</td>
<td>172.5</td>
<td>145.1, 200.8</td>
</tr>
<tr>
<td>Sleep duration (h/d)</td>
<td>8.3</td>
<td>8.0, 8.6</td>
<td>8.8</td>
<td>8.3, 9.2</td>
</tr>
<tr>
<td>BMI SDS§</td>
<td>1.2</td>
<td>0.7, 1.7</td>
<td>0.1</td>
<td>–0.7, 0.7</td>
</tr>
<tr>
<td>WHtR</td>
<td>0.5</td>
<td>0.4, 0.5</td>
<td>0.4</td>
<td>0.4, 0.4</td>
</tr>
</tbody>
</table>

MVPA, moderate-to-vigorous physical activity; SDS, standard deviation score; WHtR, waist-to-height ratio.  
* Median (25th, 75th percentile).  
† P_trend was calculated using age- and sex-adjusted linear regression models.  
‡ Mean (Min–Max).  
§ Based on age- and sex-specific median, coefficient of variation and measure of skewness values of the national German reference(36).
Table 3. Characteristics for young adulthood across categories of the adolescent lifestyle score* (Median and percentiles; mean values and minimum–maximum values)

<table>
<thead>
<tr>
<th>Lifestyle score categories (points)</th>
<th>n</th>
<th>Age at blood withdrawal (years)</th>
<th>Time span between exposure – outcome assessment (years)</th>
<th>BMI (kg/m²)</th>
<th>Waist circumference (cm)</th>
<th>TAG (mmol/l)</th>
<th>ALT (UI/l)</th>
<th>AST (UI/l)</th>
<th>GGT (UI/l)</th>
<th>HSI (total sample)</th>
<th>FLI (total sample)</th>
<th>Men (n 124)</th>
<th>Women (n 116)</th>
<th>FLI (total sample)</th>
<th>Women (n 116)</th>
<th>Men (n 124)</th>
<th>Women (n 116)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>30</td>
<td>18.4, 18.1, 22.0</td>
<td>5.8, 5.1, 9.1</td>
<td>24.8</td>
<td>81.6</td>
<td>90.5</td>
<td>22.5</td>
<td>22.5</td>
<td>15.0</td>
<td>34.4</td>
<td>35.2</td>
<td>33.4</td>
<td>35.0</td>
<td>63.0</td>
<td>79.0</td>
<td>13.0</td>
<td>30.5</td>
</tr>
<tr>
<td>2.5–7.5</td>
<td>101</td>
<td>21.1, 18.1, 24.6</td>
<td>22.4, 20.1, 25.0</td>
<td>72.7</td>
<td>70.0, 82.7</td>
<td>79.0</td>
<td>19.0</td>
<td>22.0</td>
<td>14.0</td>
<td>31.6</td>
<td>31.8</td>
<td>30.6</td>
<td>31.1</td>
<td>6.7</td>
<td>6.7</td>
<td>13.5</td>
<td>31.8</td>
</tr>
<tr>
<td>3–9</td>
<td>96</td>
<td>21.0, 18.1, 24.1</td>
<td>22.0, 19.9, 23.4</td>
<td>74.2</td>
<td>70.0, 79.5</td>
<td>79.5</td>
<td>19.0</td>
<td>22.0</td>
<td>14.0</td>
<td>30.3</td>
<td>30.2</td>
<td>30.6</td>
<td>30.5</td>
<td>6.7</td>
<td>6.7</td>
<td>13.5</td>
<td>31.8</td>
</tr>
<tr>
<td>≥4</td>
<td>13</td>
<td>21.0, 18.1, 24.2</td>
<td>22.2, 21.4, 23.2</td>
<td>78.7</td>
<td>72.5, 81.2</td>
<td>82.0</td>
<td>20.0</td>
<td>26.0</td>
<td>20.0</td>
<td>30.7</td>
<td>29.3</td>
<td>32.6</td>
<td>30.5</td>
<td>8.5</td>
<td>8.5</td>
<td>15.0</td>
<td>31.9</td>
</tr>
<tr>
<td>P&lt;sub&gt;p&lt;sub&gt;mult†&lt;/sub&gt;&lt;/sub&gt;</td>
<td>0.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.26</td>
<td></td>
<td></td>
<td></td>
<td>0.0009</td>
<td>0.0001</td>
<td>0.32</td>
<td>0.0001</td>
<td>0.0017</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

ALT, alanine-aminotransferase; AST, aspartate-aminotransferase; GGT, γ-glutamyltransferase; HSI, hepatic steatosis index; FLI, fatty liver index.

† P<sub>p<sub>mult†</sub></sub> was calculated using age- and sex-adjusted linear regression models.

Table 4. Associations between adolescence lifestyle score and fatty liver indices in young adulthood* (β-estimates and 95 % confidence intervals)

<table>
<thead>
<tr>
<th>n</th>
<th>β-estimate</th>
<th>95 % CI</th>
<th>P†</th>
<th>β-estimate</th>
<th>95 % CI</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>HSI</td>
<td>240</td>
<td>−6.6</td>
<td>−9.1, −4.0</td>
<td>&lt;0.0001</td>
<td>−5.8</td>
</tr>
<tr>
<td></td>
<td>FLI</td>
<td>240</td>
<td>−34.1</td>
<td>−44.2, −22.3</td>
<td>&lt;0.0001</td>
<td>−32.4</td>
</tr>
<tr>
<td>Men</td>
<td>HSI</td>
<td>124</td>
<td>−8.5</td>
<td>−12.1, −4.8</td>
<td>&lt;0.0001</td>
<td>−7.8</td>
</tr>
<tr>
<td></td>
<td>FLI</td>
<td>124</td>
<td>−43.5</td>
<td>−55.0, −29.0</td>
<td>&lt;0.0001</td>
<td>−42.0</td>
</tr>
<tr>
<td>Women</td>
<td>HSI</td>
<td>116</td>
<td>−3.5</td>
<td>−7.1, 0.1</td>
<td>0.06</td>
<td>−2.6</td>
</tr>
<tr>
<td></td>
<td>FLI</td>
<td>116</td>
<td>−16.9</td>
<td>−34.7, 5.7</td>
<td>0.13</td>
<td>−14.5</td>
</tr>
</tbody>
</table>

HSI, hepatic steatosis index; FLI, fatty liver index.

† Log-transformed β values were back-transformed.

‡ Adjusted for age and sex (for overall sample analyses only).§ Adjusted for age, sex, and maternal overweight.

To the best of our knowledge, there is no previous epidemiological study investigating this hypothesis in a longitudinal design covering associations in adolescence and young adulthood. Studies in adults combined between four and six lifestyle factors, whereby all studies included anthropometric markers (BMI or waist circumference), PA, smoking, and diet or dietary factors. One study additionally included sleeping habits and anxiety neurosis. Results from these studies generally support the hypothesis of an inverse association and are further confirmed and extended by our results. Nevertheless, a certain inconsistency, for example, with regard to sex differences, remained in the existing studies as well as in our results.

Studies already linking multiple lifestyle factors to NAFLD in children and adolescence are lifestyle interventions for participants with pre-existing NAFLD. The majority of studies (89 %) included a combination of physical activity and diet as intervention. Results of the meta-analysis indicated that a balanced diet combined with regular physical activity significantly improved BMI, aspartate-aminotransferase as well as alanine-aminotransferase levels and reduced the risk of steatosis by 61 %. Nevertheless, further research on the intensity and duration of physical activity is needed as well as how the risk of steatosis will change if lifestyle interventions do not persist after termination of the study.

HSI and FLI were commonly used to identify the presence of NAFLD and both have been reported as valid measures. NAFLD prevalence worldwide is higher in men than in women, which was also reflected in our current analysis.
Our results showed inverse associations in the overall sample as well as in men, whereby the $\beta$ estimates of linear regression models were consequently lower in men than in women. The association in the overall population was mainly driven by men. Previous studies showed inconsistent results with regard to sex differences in liver fat content determined by imaging techniques\(^56,57\). Nevertheless, considering the existing sex-specific differences in metabolism\(^55,58\), it seems reasonable that these differences also exist during the pathogenesis of the disease. Future research should carefully pay attention to sex-specific differences among participants.

Previous results of the DONALD study indicated that the intake of flavonoids from fruits and vegetables was inversely associated with fatty liver indices\(^59\) and that large amounts of high glycaemic carbohydrates in the evening might increase fatty liver values\(^60\). Furthermore, no association was found between fructose intake and fatty liver indices in adulthood\(^61\). The age of the considered study populations at fatty liver indices measurement during adulthood was very young (around 21 years) indicating that lifestyle in young age can have an influence on early development of liver steatosis.

So far, a uniform definition of a lifestyle score does not exist\(^62\). We have attempted to develop a lifestyle score combining evidence for single lifestyle factors dietary intake\(^20,22\), physical activity\(^27\), sedentary behaviour\(^20\), sleep duration\(^32\) and BMI\(^31\). We decided to include BMI as part of our lifestyle score, even though BMI might be a consequence of unhealthy eating habits and a predominant sedentary lifestyle rather than a genuine lifestyle factor. The results of our study showed that the significant associations between lifestyle and fatty liver indices are partly due to the inclusion of BMI. However, when we replaced BMI SDS with waist-to-height ratio, the effect sizes remained similar (online Supplementary Table S2) and when we removed BMI SDS from the lifestyle score, the effect sizes weakened but the association remained significant (online Supplementary Table S3).

Our analysis is characterised by several strengths, including the design of the study, which allowed repeated lifestyle measurements during adolescence and follow-up of individuals into adulthood. The score was created based on recommendations for absolute dietary intake, which we collected using 3-d weighed dietary record, a self-reported but valid instrument for the quantitative intake assessment. Sensitivity analyses without participants who potentially underreported their energy intake led to similar results, underlining the validity of the 3-d weighed dietary record. In addition, we used a validated questionnaire for MVPA assessment\(^35\). Lastly, we used reference-based cut-offs\(^56,98-43\) for all lifestyle factors to develop a score which can easily be adopted by others and compared across studies.
However, we acknowledge several limitations. For some participants (7.9%), only a single measurement of all lifestyle factors was available, which is not meaningful to describe participants’ habitual lifestyle. Nevertheless, we were able to show similar results in a sensitivity analysis with participants who had at least two lifestyle measurements (n = 221) (online Supplementary Table S4). It might be useful to consider daily screen time as a sum of TV, computer and smartphone, but this information was not collected. Standardised questionnaires on self-reported behaviours (sedentary behaviour and sleep duration) were not validated but used in a nationwide cohort[53]. We were not able to investigate properly the question of how many lifestyle factors were sufficient to prevent NAFLD. In addition, the generalisability of our results is limited because participants in the DONALD study are characterised by a high socio-economic status. Nevertheless, the dietary intake and activity behaviour do not differ from representative other German studies[60]. Confounding by unmeasured covariates, such as family history of diabetes or menstruation status, might remain.

In conclusion, our data suggest that a healthy lifestyle (healthy diet, high PA level, low sedentary behaviour, age-appropriate sleep duration and healthy body weight) during adolescence is associated with lower fatty liver indices in young adulthood, predominately in men. We were the first analysing repeated lifestyle measurements in adolescence as risk factors for higher fatty liver indices in early adulthood. To confirm and extend our findings, further analyses across the life course in larger study populations are needed which might also be able to take later disease incidence into account. The type and extent of relevant lifestyle factors also warrant further in-depth analysis.

Acknowledgements

The authors would like to thank the staff of the DONALD study for carrying out all lifestyle measurements as well as all study participants and their families for the provision of their data.

This work was supported by Diet-Body–Brain (DietBB), the Competence Cluster in Nutrition Research funded by the Federal Ministry of Education and Research (FKZ: 01EA1410A). The DONALD study is supported by the Ministry of Science and Research of North Rhine Westphalia, Germany. The German Diabetes Center is supported by the Ministry of Culture and Science of the State of North Rhine-Westphalia and the German Federal Ministry of Health. This study was supported in part by a grant from the German Federal Ministry of Education and Research to the German Center for Diabetes Research (DZD).


C. H. received a research grant from Sanofi–Aventis outside the submitted work. MR is on scientific advisory boards of Allergan, Eli Lilly, Novartis, Novo Nordisk, Pfizer, Sanofi, Target NRW and Terra Firma and has received support for investigator-initiated studies from Boehringer Ingelheim, Nutricia/Danone and Sanofi–Aventis. The other authors declare no conflict of interest.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114522001313

References


50. Lonardo A, Nascimbeni F, Ballestri S, et al. (2019) Sex differences in nonalcoholic fatty liver disease: state of the art?


