Tracking of secretory phospholipase A2 enzyme activity levels from childhood to adulthood: a 21-year cohort

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Abstract
Objective: Secretory phospholipase A2 (sPLA2) enzyme activity is a potential inflammatory biomarker for cardiovascular disease. We examined the tracking, or persistence, of sPLA2 enzyme activity levels from childhood to adulthood, and identify potentially modifiable factors affecting tracking.
Method: Prospective cohort of 1735 children (45% females) who had serum sPLA2 enzyme activity levels and other cardiovascular disease risk factors measured in 1980 that were followed-up in 2001.
Results: sPLA2 activity tracked from childhood to adulthood for males ($r = 0.39$) and females ($r = 0.45$). Those who decreased body mass index relative to their peers were more likely to resolve elevated childhood sPLA2 levels than have persistent elevated sPLA2 levels in childhood.

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and adulthood. Those who consumed less fruit, and gained more body mass index relative to their peers, began smoking or were a persistent smoker between childhood and adulthood were more likely to develop incident elevated sPLA2 levels than those with persistent not elevated sPLA2 levels.

Conclusions: Childhood sPLA2 enzyme activity levels associate with adult sPLA2 levels 21 years later. Healthful changes in modifiable risk factors that occur between childhood and adulthood might prevent children from developing elevated sPLA2 levels in adulthood.

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Monitoramento dos níveis de atividade da enzima fosfolipase A2 secretória da infância à vida adulta: uma coorte de 21 anos

Resumo

Objetivo: A atividade da enzima fosfolipase A2 secretória (sPLA2) é um possível biomarcador inflamatório de doença cardiovascular. Examinamos o monitoramento, ou a persistência, dos níveis de atividade da enzima sPLA2 da infância à vida adulta e identificamos fatores possivelmente modificáveis que afetam o monitoramento.

Método: Coorte prospectiva de 1.735 crianças (45% do sexo feminino) cujos níveis de atividade da enzima sPLA2 no soro e outros fatores de risco para doença cardiovascular foram medidos em 1980 e acompanhados até 2011.

Resultados: Atividade da enzima sPLA2 monitorada da infância à vida adulta para indivíduos do sexo masculino (r = 0.39) e sexo feminino (r = 0.45). Aqueles que diminuíram seus índices de massa corporal com relação a seus pares foram mais propensos à redução dos níveis elevados de sPLA2 na infância do que a manter níveis persistentemente elevados de sPLA2 na infância e vida adulta. Aqueles que consumiram menos frutas e ganharam mais índice de massa corporal com relação a seus pares, que começaram a fumar ou foram fumantes persistentes entre a infância e vida adulta foram mais propensos a desenvolver níveis de sPLA2 elevados do que aqueles com níveis de sPLA2 não elevados persistentes.

Conclusões: Os níveis de atividade da enzima sPLA2 na infância estão associados aos níveis de sPLA2 na vida adulta, 21 anos mais tarde. As mudanças saudáveis nos fatores de risco modificáveis que ocorrem entre a infância e a vida adulta podem evitar que as crianças desenvolvam níveis elevados de sPLA2 na vida adulta.

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Methods

Study population

The Cardiovascular Risk in Young Finns Study is a population-based prospective follow-up on 3596 Finnish children and adolescents aged 3–18 years who participated at baseline in 1980. In 2001, 2283 of the baseline participants attended clinics, aged 24–39 years. Measurements of serum sPLA2 were performed on those with available samples from both 1980 and 2001 (n = 2245). We excluded participants who had a condition that may predispose them to altered sPLA2 levels: participants with chronic rheumatic disease (n = 34; sPLA2 activity 1.80 (1.29–2.22) nmol/min/mL in 2001); females who reported they were pregnant (n = 59; sPLA2 activity 1.67 (1.33–2.08) nmol/min/mL in 2001); participants who reported having an infection with fever in the last 2 weeks (n = 104; sPLA2 activity 1.65 (1.32–2.08) nmol/min/mL in 2001); females who reported current use of oral contraceptives (n = 288; sPLA2 activity 1.78 (1.39–2.28) nmol/min/mL in 2001; n = 25; sPLA2 activity 0.52 (0.41–0.97) nmol/min/mL in 1980). After exclusions, data on 1735 participants were left for our primary analyses. The study was conducted according to the Declaration of Helsinki, and local ethics committees approved the study protocols. Written informed consent was obtained from all participants in 2001, and their parents in 1980.

Clinical measurements and risk factors

At both time-points, height was measured with a Seca anthropometer and weight was measured with Seca scales (Vogel Halke, Hamburg, Germany). Body mass index (BMI, kg/m²) was calculated. Self-report questionnaires administered at baseline and follow-up collected data on smoking, dietary habits, and physical activity. Information on smoking habits was only collected amongst participants aged 12–18 years at baseline. Participants who smoked at least weekly at baseline or daily at follow-up were considered smokers. Fruit and vegetable consumption was reported based on six options (1 = daily, 2 = almost every day, 3 = a couple of times per week, 4 = about once a week, 5 = a couple of times per month, 6 = less than a couple of times per month), which was converted into times of consumption per week (1 = 9.5, 2 = 6.3, 3 = 3.3, 4 = 1.2, 5 = 0.3, 6 = 0.1). Physical activity index (range from 5 (low) to 15 (high)), was determined by participant’s self-report of duration, intensity, and frequency of physical activity. Socioeconomic position (SEP) was based on highest level of parental occupation at baseline (manual, lower-grade non-manual, higher-grade non-manual) and participant occupation at follow-up.

Serum sPLA2 enzyme activity measurements

In 1980 and 2001, blood samples were taken from the antecubital vein after participants confirmed they had fasted overnight. Serum sPLA2 enzyme activity was measured in 2006 from 1980 blood samples stored at −20°C, and 2001 samples stored at −80°C. Measurement of sPLA2 enzyme activity includes several groups of sPLA2 (Ia, V, X) expressed in atherosclerotic lesions from human and animal models. Hydrolysis of substrate without plasma was used as the negative control and deduced from sPLA2 activity. Samples were tested in duplicate. Intra- and inter-assay coefficients of variation were <10% and the minimum detectable level of sPLA2 activity was 0.10 nmol/min/mL.

Statistical analyses

All analyses were performed using STATA 13.1 (StataCorp, College Station, TX, USA).

Demographics

Baseline and follow-up characteristics are displayed for male and female participants. Continuous variables are displayed as mean (standard deviation) for normally distributed variables and median (25–75th percentiles) for variables with a non-normal distribution. Categorical variables are displayed as N (percent). We also plot age on mean sPLA2 enzyme activity levels by sex.

Tracking of sPLA2 levels from childhood to adulthood

Tracking was estimated by two approaches used in previous tracking studies: (1) rank correlations by applying Pearson’s correlation to the rank of baseline and follow-up sPLA2 measurements; and (2) the proportion of participants remaining in the upper quarter of age- and sex-specific sPLA2 distributions at both time-points. We report partial correlations adjusted for age when ages are combined, or adjusted age and sex when data for age and sex are combined. sPLA2 measurements at both time-points were ranked prior to correlation analyses owing to a right-skewed distribution. We performed Pearson’s correlation on the sPLA2 ranks, as STATA does not have a function to perform partial Spearman’s correlations.

Factors affecting sPLA2 levels from child to adulthood

Participants were divided into four tracking groups based on their sPLA2 enzyme activity status from childhood to adulthood. Participants who remained in the upper quarter of age- and sex-specific sPLA2 distributions at both time-points were classified as persistent elevated; those who were in the upper quarter in childhood but not at follow-up were classified as resolution; those who were not in the upper quarter in childhood but were in adulthood were classified as incident; and those who did not have sPLA2 levels in the upper quarter at both childhood and adulthood were classified as persistent not elevated (Supplemental Fig. 1). This approach has been adopted in other studies examining factors that influence tracking of lipids and blood pressure between childhood and adulthood.

Changes (adult minus child) in continuous variables (BMI, physical activity index, fruit consumption, vegetable consumption) were analysed using age- and sex-specific z-scores at each time-point. As an indicator of change or stability
in SEP, we created a categorical social mobility variable\textsuperscript{25} that considered the highest level of parental (mother or father) occupation at baseline and participant occupation at follow-up.\textsuperscript{19} This variable has the following categories: persistently low (low at baseline and follow-up), persistently medium (medium at baseline and follow-up), persistently high (high at baseline and follow-up), upwardly mobile (moving from medium at baseline to high at follow-up, or low at baseline to medium or high at follow-up), and downwardly mobile (moving medium at baseline to low at follow-up, or from high at baseline to medium or low at follow-up). Participants were categorised into one of four categories based on their smoking status at both time-points: not smoking at either time-point, stopped smoking (smoking at baseline, not at follow-up), began smoking (did not smoke at baseline, smoked at follow-up), and smoker at both time-points.

Using logistic regression, we compared changes in the lifestyle-related variables of BMI, physical activity index, fruit consumption, vegetable consumption, smoking status, and social mobility between persistent elevated (reference) and resolution sPLA2 tracking groups and between persistent not elevated (reference) and incident sPLA2 tracking groups. As no significant sex interactions were observed, the data are not stratified by sex.

**Results**

**Demographics**

Characteristics outlining the population are presented in **Table 1**. At baseline and follow up, females had higher sPLA2 enzyme activity levels compared with males. \textbf{Fig. 1} displays mean sPLA2 enzyme activity levels as a function of age at both time-points. There was a significant (negative) age trend for boys aged 3–18 years ($p < 0.001$). There were no clear age trends in girls, or in adulthood for males or females.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N^a$ Statistic</td>
<td>$N^a$ Statistic</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Childhood</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>962</td>
<td>10.7 (5.0)</td>
<td>773</td>
<td>11.3 (4.8)</td>
</tr>
<tr>
<td>sPLA2 activity, nmol/mL/min</td>
<td>962</td>
<td>0.57 (0.37–0.84)</td>
<td>773</td>
<td>0.72 (0.48–1.02)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>957</td>
<td>144.6 (27.5)</td>
<td>773</td>
<td>143.7 (22.9)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>957</td>
<td>40.7 (20.0)</td>
<td>771</td>
<td>39.5 (16.1)</td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>956</td>
<td>18.0 (3.2)</td>
<td>771</td>
<td>18.1 (3.0)</td>
</tr>
<tr>
<td>Physical activity index\textsuperscript{a}</td>
<td>643</td>
<td>9.6 (1.9)</td>
<td>545</td>
<td>8.6 (1.6)</td>
</tr>
<tr>
<td>Fruit consumption, serves/wk</td>
<td>950</td>
<td>6.7 (2.9)</td>
<td>767</td>
<td>6.9 (2.8)</td>
</tr>
<tr>
<td>Vegetable consumption, serves/wk</td>
<td>949</td>
<td>6.1 (2.9)</td>
<td>767</td>
<td>6.3 (2.8)</td>
</tr>
<tr>
<td>Currently smoking ≥ once/week\textsuperscript{c}</td>
<td>466</td>
<td>71 (15.2)</td>
<td>431</td>
<td>44 (10.2)</td>
</tr>
<tr>
<td>Parental occupational status</td>
<td>943</td>
<td></td>
<td>756</td>
<td></td>
</tr>
<tr>
<td>Manual</td>
<td>396 (42.0)</td>
<td>321 (42.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower non-manual</td>
<td>378 (40.1)</td>
<td>311 (41.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper non-manual</td>
<td>169 (17.9)</td>
<td>124 (16.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Adulthood</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>959</td>
<td>31.7 (5.0)</td>
<td>772</td>
<td>32.3 (4.8)</td>
</tr>
<tr>
<td>sPLA2 activity, nmol/mL/min</td>
<td>962</td>
<td>1.37 (1.10–1.69)</td>
<td>773</td>
<td>1.61 (1.30–2.00)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>956</td>
<td>179.4 (6.5)</td>
<td>768</td>
<td>165.9 (5.9)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>957</td>
<td>83.0 (14.7)</td>
<td>768</td>
<td>67.9 (14.0)</td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>956</td>
<td>25.8 (4.1)</td>
<td>768</td>
<td>24.7 (4.9)</td>
</tr>
<tr>
<td>Physical activity index</td>
<td>899</td>
<td>9.9 (2.5)</td>
<td>716</td>
<td>9.9 (2.2)</td>
</tr>
<tr>
<td>Fruit consumption, serves/wk</td>
<td>934</td>
<td>5.0 (3.1)</td>
<td>748</td>
<td>6.3 (3.0)</td>
</tr>
<tr>
<td>Vegetable consumption, serves/wk</td>
<td>930</td>
<td>4.9 (3.1)</td>
<td>741</td>
<td>6.1 (3.1)</td>
</tr>
<tr>
<td>Currently smoking daily</td>
<td>932</td>
<td>281 (30.2)</td>
<td>757</td>
<td>139 (18.4)</td>
</tr>
<tr>
<td>Participant’s occupational status</td>
<td>814</td>
<td></td>
<td>675</td>
<td></td>
</tr>
<tr>
<td>Manual</td>
<td>353 (43.4)</td>
<td>163 (24.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower non-manual</td>
<td>222 (27.3)</td>
<td>389 (57.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper non-manual</td>
<td>239 (29.4)</td>
<td>123 (18.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistics are mean (SD) or median (25th, 75th percentiles) for continuous variables or $N$ (percent) for categorical variables.

\textsuperscript{a} Changing $N$s are the result of missing data for some variables.

\textsuperscript{b} Only participants aged 9 years or older at baseline had physical activity data collected.

\textsuperscript{c} Only participants aged 12 years or older at baseline had smoking data collected.
Factors affecting sPLA2 levels from youth to adulthood

Table 2 shows the association of changes in different lifestyle factors on the tracking of sPLA2 enzyme activity groups. Compared with those in the persistent elevated group, the resolution group had decreased their BMI relative to peers who had experienced a relative increase in BMI.

Comparing incident and persistent not elevated sPLA2 enzyme activity groups, those in the incident group tended to have gained more BMI and decreased fruit consumption compared with those in the persistent not elevated group, who had maintained stable BMI and had increased fruit consumption relative to their peers. Those in the incident group were also more likely to have begun smoking since childhood (21.1% vs. 15.3%), or were a smoker at both time-points (11.9% vs. 5.0%).

Discussion

sPLA2 is a marker of vascular inflammation that might have a role in the pathogenesis of atherosclerosis. Since exposure to risk factors in childhood contributes to atherosclerosis later in life, we examined the ability to predict adult sPLA2 levels from measures collected in childhood (tracking). We showed that sPLA2 enzyme activity in childhood correlates with levels measured 21 years later in adulthood and that approximately 40% of children with high levels maintain this level to adulthood. Furthermore, our findings suggest that healthy improvements in the modifiable risk factors of fruit consumption, smoking status, and BMI have the potential to decrease the development of an elevated sPLA2 level in adulthood.

As no previous studies have examined the tracking of sPLA2 levels spanning childhood to adulthood, we provide comparison with tracking of other CVD risk factors reported in earlier findings from the Cardiovascular Risk in Young Finns Study (Supplemental Table 3). On the basis of rank correlations, sPLA2 enzyme activity tracked similarly compared with other established risk factors (lipoproteins, BMI, systolic blood pressure). Of interest, sPLA2 enzyme activity tracked more strongly than high-sensitivity C-reactive protein (hsCRP, \( r = 0.43 \) vs. \( r = 0.29 \)), an inflammatory biomarker linked to atherosclerosis and CVD. Previously, we have found child lipoproteins, blood pressure, and BMI to predict adult carotid intima-media thickness (cIMT), an established preclinical marker of atherosclerosis, independent of adult levels of the same risk factors. However, child hsCRP was not associated with adult cIMT. Should a causal role of sPLA2 in preclinical atherosclerosis be confirmed, and if sPLA2 enzyme activity tracks as our data suggest, future studies could examine the utility of child sPLA2 enzyme activity levels to predict adult markers of atherosclerosis independent of other risk factors.

In the absence of support from clinical trials spanning multiple decades, our data also suggest positive changes made to lifestyle-related risk factors in the time between childhood and adulthood might help individuals to improve
<table>
<thead>
<tr>
<th>Factor</th>
<th>Persistent elevated</th>
<th>Resolution</th>
<th>Incident</th>
<th>Persistent not elevated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Δ Body mass index z-score</strong></td>
<td>N 168</td>
<td>N 258</td>
<td>N 218</td>
<td>N 1072</td>
</tr>
<tr>
<td></td>
<td>Statistic&lt;sup&gt;a&lt;/sup&gt; 0.17 (1.17)</td>
<td>Statistic&lt;sup&gt;a&lt;/sup&gt; -0.01 (0.92)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Statistic&lt;sup&gt;a&lt;/sup&gt; 0.14 (0.98)</td>
<td>Statistic&lt;sup&gt;a&lt;/sup&gt; 0.00 (0.95)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Δ Physical activity z-score</strong></td>
<td>N 107</td>
<td>N 164</td>
<td>N 150</td>
<td>N 681</td>
</tr>
<tr>
<td></td>
<td>Statistic&lt;sup&gt;a&lt;/sup&gt; 0.15 (1.18)</td>
<td>Statistic&lt;sup&gt;a&lt;/sup&gt; 0.04 (1.17)</td>
<td>Statistic&lt;sup&gt;a&lt;/sup&gt; -0.03 (1.21)</td>
<td>Statistic&lt;sup&gt;a&lt;/sup&gt; -0.05 (1.21)</td>
</tr>
<tr>
<td><strong>Δ Fruit consumption z-score</strong></td>
<td>N 167</td>
<td>N 250</td>
<td>N 214</td>
<td>N 1034</td>
</tr>
<tr>
<td></td>
<td>Statistic&lt;sup&gt;a&lt;/sup&gt; -0.13 (1.25)</td>
<td>Statistic&lt;sup&gt;a&lt;/sup&gt; -0.01 (1.30)</td>
<td>Statistic&lt;sup&gt;a&lt;/sup&gt; -0.17 (1.21)</td>
<td>Statistic&lt;sup&gt;a&lt;/sup&gt; 0.07 (1.33)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Δ Vegetable consumption z-score</strong></td>
<td>N 166</td>
<td>N 250</td>
<td>N 213</td>
<td>N 1023</td>
</tr>
<tr>
<td></td>
<td>Statistic&lt;sup&gt;a&lt;/sup&gt; 0.08 (1.32)</td>
<td>Statistic&lt;sup&gt;a&lt;/sup&gt; 0.17 (1.32)</td>
<td>Statistic&lt;sup&gt;a&lt;/sup&gt; -0.04 (1.22)</td>
<td>Statistic&lt;sup&gt;a&lt;/sup&gt; 0.01 (1.28)</td>
</tr>
</tbody>
</table>

**Smoking status, %**

- Not smoking at either time-point: N 58, Statistic<sup>a</sup> 69.9
- Stopped smoking: N 4, Statistic<sup>a</sup> 48.0
- Began smoking: N 13, Statistic<sup>a</sup> 15.7
- Smoker at both time-points: N 8, Statistic<sup>a</sup> 9.6

**Social mobility, %**

- Persistently low: N 27, Statistic<sup>a</sup> 18.9
- Persistently moderate: N 26, Statistic<sup>a</sup> 18.2
- Persistently high: N 10, Statistic<sup>a</sup> 7.0
- Downwardly mobile: N 31, Statistic<sup>a</sup> 21.7
- Upwardly mobile: N 49, Statistic<sup>a</sup> 34.4

Totals for percentages may not add to 100 because of rounding.

<sup>a</sup> Statistics are z-scores for continuous variables or proportions for categorical variables.

<sup>b</sup> p < 0.05 for comparisons between incident and persistent not elevated (reference group) tracking groups.

<sup>c</sup> p ≤ 0.10 for comparisons between incident and persistent not elevated (reference group) tracking groups or resolution and persistent elevated (reference group) tracking groups.

This table presents factors affecting tracking of secretory phospholipase A2 (sPLA2) enzyme activity from childhood (1980) to adulthood (2001) in the Cardiovascular Risk in Young Finns Study.

The data show that factors such as body mass index, physical activity, fruit consumption, and smoking status are associated with sPLA2 enzyme activity. A higher body mass index, lower physical activity, and higher fruit consumption are associated with lower sPLA2 enzyme activity. Smoking status also plays a significant role, with those who stop smoking having a lower sPLA2 enzyme activity compared to those who continue to smoke.

The table also includes data on social mobilityVisits the website of each institution to gather information on their research interests and faculty members. The data show that those who remain in the same social mobility category over time have a lower sPLA2 enzyme activity compared to those who experience upward or downward mobility.

The study’s limitations include the use of serum samples, which may not represent the current sPLA2 levels due to storage and handling differences. Moreover, the study’s duration (35 years) may introduce additional variables not accounted for. Despite these limitations, the study provides valuable insights into the long-term tracking of sPLA2 enzyme activity and its associations with various factors.
examine similar aims. The large sample size, long duration of follow-up, with participants of different social backgrounds and who are well phenotyped allows our findings to be generalised at a population level and to infer long-term estimates of effect. 31

Our findings show that childhood elevations in sPLA2 levels associate with elevated sPLA2 levels in adulthood. Further, our data suggest that intervention and prevention programmes that aim to improve conventional modifiable risk factors for CVD by advocating for not smoking, higher fruit consumption, and reduced excess adiposity in the time between childhood and adulthood might influence whether individuals maintain, develop, or resolve elevated child sPLA2 levels.

Funding

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Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jped.2018.01.002.

References


