Decreased prevalence of hypercholesterolaemia and stabilisation of obesity trends in 5-year-old children: possible effects of changed public health policies

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Abstract

Background: Overweight/obesity in children is a worldwide public health problem. Together with hypercholesterolaemia they are associated with early atherosclerotic complications.

Objectives: In this study, we aimed to investigate the anthropometric characteristics and total cholesterol (TC) levels in a population of 5-year-old children, to determine trends in the prevalence of overweight/obesity and hypercholesterolaemia in 5-year-old children over a period of 8 years (2001–2009) and to assess the impact of modified national nutritional guidelines for kindergartens implemented in 2005.


Subjects: Altogether, 12 832 (6308 girls/6524 boys) children were included.

Methods: Overweight/obesity was defined by IOTF criteria. Hypercholesterolaemia was defined by TC level > 5 mmol/l. Multivariable logistic regression models were used.

Results: No correlation between BMI values and TC levels was found. Overweight and obesity prevalence were stabilised from 2001 to 2009 (odds ratio (OR) (95% CI): 1.13 (0.99–1.3) and 1.13 (0.89–1.42) respectively). Girls were more frequently overweight/obese than boys (OR (95% CI): 0.71 (0.65–0.79) and 0.75 (0.64–0.89) respectively). Prevalence of hypercholesterolaemia significantly decreased from 2001 to 2009 (OR (95% CI): 0.47 (0.41–0.55)). It was less frequent in boys than in girls (OR (95% CI): 0.7 (0.61–0.8)).

Conclusions: This is the first study to describe a negative trend in the prevalence of hypercholesterolaemia in pre-pubertal children. In addition, the prevalence of overweight/obesity in these children has been stabilised. Nationwide changes in public health policies could have influenced these observations.

Introduction

Obesity in children is a major health problem of global significance (1, 2). Until very recently the prevalence was increasing. However, in the last couple of years several studies from various countries worldwide reported that the prevalence of childhood obesity is reaching a plateau or is even slightly decreasing (3, 4). Nevertheless, it is still too high. Complications of obesity, such as hypertension, impaired glucose regulation, fatty liver, systemic...
inflammation and premature signs of atherosclerosis, are already identified in childhood. Furthermore, obese children have a higher prevalence of dyslipidaemia (5), and the prevalence of metabolic syndrome in children is increasing with the degree of obesity (6, 7). Obese children are at greater risk of becoming obese adults and prematurely develop cardiovascular diseases (CVD) (8).

Hypercholesterolaemia in children is another risk factor for early development of atherosclerosis and CVD (9). Hypercholesterolaemia in children most often has a polygenic aetiology. Familial hypercholesterolaemia (FH) with an incidence of 1/500 is the most common primary dyslipidaemia in childhood (10). Primary CVD prevention measures similar to those in obesity are crucial, including strategies to identify individuals at high risk (e.g. children with FH, children with morbid obesity), and population-based strategies to reduce rates of hypercholesterolaemia and/or obesity (e.g. promotion of healthy diet, physical activity, etc.) (11).

Slovenia is—according to the available data—currently the only country worldwide with the universal screening for hypercholesterolaemia, which was introduced as a part of mandatory nationwide medical examination in the year 1995 for 5-year-old children (12). The decision to introduce a universal general screening in children at this age was based on the fact that the process of atherosclerosis starts early in the childhood, cholesterol levels can be discriminated best between people with and without FH at the age of 1–9 years, dyslipidaemia in children is virtually asymptomatic and at the age of 5 years nutritional intervention can already be advised (12, 13, 14). Only recently, a universal screening for hypercholesterolaemia in pre-pubertal children was recommended by the National Lipid Association in the USA, as a preferred method of screening for hypercholesterolaemia in primary prevention efforts (15).

Previous nationally representative studies on obesity prevalence and total cholesterol (TC) levels in the Slovenian 5-year-old children were performed in the years 2001 and 2003–2005 (overweight and obesity only) (16, 17). In the year 2005, new national nutritional guidelines for the preparation of meals in kindergartens and schools were adopted in Slovenia, advocating decreased total fat and carbohydrate intake (18). As over 75% of the Slovenian 5-year-old children visit kindergarten (19), the new guidelines could have had some population-wide impact.

The main objectives of this study were to investigate the anthropometric characteristics and TC levels in a population of 5-year-old children and to determine trends in the prevalence of overweight/obesity and of hypercholesterolaemia in 5-year-old children over a period of 8 years (2001–2009), which could reflect new nutritional guidelines for kindergartens adopted in the year 2005.

Subjects and methods

Data were obtained from 5-year-old children on the occasion of a mandatory nationwide medical examination. Children were formally invited to attend the examination, as a rule close to their fifth birthday, by their chosen medical care provider in their local medical institution. During the examination blood collection (venous blood in EDTA) for the determination of blood count and TC was carried out. Children did not need to be in a fasted state. TC value <5 mmol/l was considered normal. If a TC value between 5.0 and 6.0 mmol/l was determined, a fasting lipid profile was performed (TC, LDL, HDL and triglycerides) (12, 20). However, as the screening data on TC were collected anonymously, follow-up fasting lipid profiles were not available.

Altogether, 12 832 children from all parts of the country were included in the study. For the purpose of the study, Slovenia was divided into 12 regions of residency. First, data from 5406 children (49% girls and 51% boys) examined in year 2001 were analysed. Furthermore, to determine trends for the period 2001–2009, also data from 2742 randomly selected children (48% girls and 52% boys) examined in year 2001 and data from 4684 children (49% girls and 51% boys) examined in years 2003–2005 (overweight and obesity data only) were reanalysed, enabling comparisons (16, 17). The study was approved by the National Medical Ethics Committee (#25/10/09).

Anthropometric characteristics

The height and weight were measured by a trained nurse using validated stadiometers and electronic digital scales. Both were rounded to the tenth decimal place. BMI was calculated as weight (kg) divided by squared height (m²).

Overweight and obesity were defined by the IOTF criteria (21) corresponding to adult’s BMI cut-offs of 25 and 30 kg/m² at 18 years of age. These criteria define age- and sex-specific cut-off points from 2- to 18-year-old children.

Laboratory methods

TC level was determined by cholesterol-oxidase/p-aminofenazon (CHOD-PAP) method in the laboratories.
of participating primary health care facilities, according to manufacturer’s instructions. The fact that this method remained unchanged throughout the study was ascertained by a questionnaire. TC levels >5 mmol/l were considered elevated (22).

Statistical analysis

Data from descriptive statistics were reported as numbers and percentages of subjects who were overweight (including the obese), obese or had hypercholesterolaemia (with its 95% CI), separately for males and females. Numerical variables were summarised as means (interquartile range). Mean TC levels were compared using Welch two-sample t-test and reporting 95% CI for the mean differences. Multivariable logistic regression models were used to investigate the association between being overweight and sex, calendar year of the survey and region of residence; an interaction term between sex and year was also included in the models. Results were reported as odds ratio (OR) with their 95% CI and P values. The statistical significance of the interaction between sex and calendar year was tested using the likelihood ratio test; non-significant interactions were removed from the models. The multivariable modelling approach was used as it allowed us to disentangle the effect of each studied variable separately. The reference value of the OR calculated for the regions was the overall average value. Similar analyses were performed using obesity and hypercholesterolaemia as outcomes. The association between BMI and TC level (used as a numerical variable) was assessed using Spearman’s correlation (r). R statistical language was used for the statistical analyses (23).

Results

After the exclusion of subjects with missing data (either weight, height or TC, altogether 278 subjects) the core dataset comprised 12 832 5-year-old children for the analysis of trends in overweight/obesity (examined in years 2001, 2003–2005 and 2009) and 7554 5-year-old children for the analysis of trends in TC levels (examined in years 2001 and 2009).

Anthropometric characteristics and TC levels

The anthropometric characteristics of the subjects and the prevalence of overweight/obesity are presented in Table 1. Mean TC level of 5-year-old children in 2009 decreased from 4.4 to 4.1 mmol/l in year 2001 (mean difference Z0.26, 95% CI: 0.23–0.30 mmol/l, P<0.001); the mean decrease was statistically significant also when girls and

**Table 1** Anthropometric data and overweight/obesity prevalence (and change in prevalence) for 5-year-old girls and boys in the period from 2001 to 2009.

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>2001</th>
<th>2004</th>
<th>2009</th>
<th>△ From 2001 % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (n)</td>
<td>1325</td>
<td>2317</td>
<td>2666</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²; median (IQR))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls</td>
<td>15.3 (14.4–16.5)</td>
<td>15.4 (14.4–16.5)</td>
<td>15.5 (14.5–16.5)</td>
<td>0.5 (−1.2 to 2.2)</td>
</tr>
<tr>
<td>Boys</td>
<td>15.4 (14.6–16.5)</td>
<td>15.4 (14.6–16.5)</td>
<td>15.5 (14.6–16.6)</td>
<td>0.5 (−1.2 to 2.2)</td>
</tr>
<tr>
<td>Overweight (n (%); 95% CI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>387/2742 (14.1); 12.9–15.5</td>
<td>684/4684 (14.6); 13.6–15.6</td>
<td>848/5406 (15.7); 14.7–16.7</td>
<td>0.5 (−1.2 to 2.2)</td>
</tr>
<tr>
<td>Girls</td>
<td>209/1325 (15.8); 13.9–17.8</td>
<td>387/2317 (16.7); 15.2–18.2</td>
<td>486/2666 (18.2); 16.8–19.7</td>
<td>0.9 (−1.6 to 3.5)</td>
</tr>
<tr>
<td>Boys</td>
<td>178/1417 (12.6); 10.9–14.4</td>
<td>297/2367 (12.6); 11.3–13.9</td>
<td>362/2740 (13.2); 12.0–14.5</td>
<td>0.0 (−2.2 to 2.2)</td>
</tr>
<tr>
<td>Obese (n (%); 95% CI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>126/2742 (4.6); 3.9–5.4</td>
<td>207/4684 (4.4); 3.9–5.1</td>
<td>283/5406 (5.2); 4.7–5.9</td>
<td>−0.2 (−1.2 to 0.8)</td>
</tr>
<tr>
<td>Girls</td>
<td>71/1325 (5.4); 4.3–6.7</td>
<td>110/2317 (4.7); 4.0–5.7</td>
<td>164/2666 (6.2); 5.3–7.1</td>
<td>0.8 (−2.2 to 0.9)</td>
</tr>
<tr>
<td>Boys</td>
<td>55/1417 (3.9); 3.0–5.0</td>
<td>97/2367 (4.1); 3.4–5.0</td>
<td>119/2740 (4.3); 3.6–5.2</td>
<td>0.2 (−1.1 to 1.6)</td>
</tr>
</tbody>
</table>

IQR, interquartile range.
boys were analysed separately (Table 2). Mean and median TC levels were very similar due to the symmetric distribution. Girls compared with boys had 0.11 mmol/l higher mean TC levels (95% CI: 0.07–0.15, \( P < 0.001 \)) in year 2009 (as compared with 0.13 mmol/l in year 2001, 95% CI: 0.07–0.19, \( P < 0.001 \)) (Table 2). Median TC levels decreased from 4.4 to 4.2 mmol/l in girls and from 4.3 to 4.0 mmol/l in boys. Ninety-fifth percentile TC levels values decreased in girls from 5.8 to 5.4 mmol/l and in boys from 5.6 to 5.3 mmol/l. BMI and TC levels were found not to be associated (\( r = 0.031 \)); the lack of association persisted when the data were analysed separately for boys (\( r = 0.06 \)) and for girls (\( r = 0.001 \)).

### Trends in overweight/obesity prevalence from 2001 to 2009

The prevalence of overweight was altogether stable (Table 1). In girls there was a positive but statistically non-significant (\( P = 0.11 \)) trend from 15.8% in 2001 to 16.7% in 2004 (0.9% difference, 95% CI: –1.6 to 3.5%) and to 18.2% in 2009 (difference from 2001: 2.4%, 95% CI: 0–5%). In boys the prevalence remained stable (\( P = 0.50 \): 12.6% in 2001, 12.6% in 2004 and 13.2% in 2009).

In the multivariable logistic model for overweight (including obesity), the interaction between sex and survey year was not statistically significant (\( P = 0.7 \)) and was removed from the model. As shown in Table 3, boys had a significantly lower probability of being overweight compared with girls and the probability of being overweight remained unchanged from 2001 to 2005, while there was a non-significant trend towards an increase in 2009.

Trends in the prevalence of obesity were similar as for overweight (Table 1). Prevalence of obesity was 5.4% in 2001, 4.7% in 2004 and 6.2% in 2009 (\( P = 0.10 \)) in girls and 3.9% in 2001, 4.1% in 2004 and 4.3% in 2009 (\( P = 0.10 \)) in boys. Results from the multivariable analysis were similar to those reported for overweight.

The interaction between sex and year was not statistically significant (\( P = 0.5 \)). As shown in Table 3, boys were less likely to be obese than girls and the probability of being obese did not change significantly from 2001 to 2005 and 2009.

### Table 2 Elevated total cholesterol (TC) level prevalence for 5-year-old boys and girls and mean values for TC according to sex in the years 2001 and 2009.

<table>
<thead>
<tr>
<th></th>
<th>2001</th>
<th>2009</th>
<th>( \Delta )</th>
<th>( \Delta 95% \text{ CI} )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls TC &gt; 5 mmol/l ((n%); 95% \text{ CI})</td>
<td>291/1325 (22.0); 14.6–18.5</td>
<td>256/2381 (10.8); 9.6–12.1</td>
<td>11.2%</td>
<td>8.6–13.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Boys TC &gt; 5 mmol/l ((n%); 95% \text{ CI})</td>
<td>233/1417 (16.4); 19.8–24.3</td>
<td>191/2431 (7.9); 6.9–9.0</td>
<td>8.6%</td>
<td>6.3–10.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC (mmol/l), mean ((95% \text{ CI}) )</td>
<td>Girls</td>
<td>4.5 (4.42–4.50)</td>
<td>4.2 (4.16–4.22)</td>
<td>0.27</td>
<td>0.22–0.32</td>
</tr>
<tr>
<td>Boys</td>
<td>4.3 (4.29–4.37)</td>
<td>4.1 (4.05–4.10)</td>
<td>0.25</td>
<td>0.20–0.30</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### Table 3 Odds ratios of being overweight, obese or have total cholesterol (TC) level >5 mmol/l and sex and survey year as determined by multivariable logistic regression models.

<table>
<thead>
<tr>
<th>Year</th>
<th>Overweight</th>
<th></th>
<th></th>
<th>Obesity</th>
<th></th>
<th></th>
<th>TC &gt; 5 mmol/l</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>( P )</td>
<td>OR</td>
<td>95% CI</td>
<td>( P )</td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>0.71</td>
<td>0.65–0.79</td>
<td>&lt;0.01</td>
<td>0.75</td>
<td>0.64–0.89</td>
<td>&lt;0.01</td>
<td>0.7</td>
<td>0.61–0.8</td>
</tr>
<tr>
<td>Year</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>1.01</td>
<td>0.87–1.18</td>
<td>0.86</td>
<td>0.95</td>
<td>0.74–1.23</td>
<td>0.72</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td>2009</td>
<td>1.13</td>
<td>0.99–1.3</td>
<td>0.08</td>
<td>1.13</td>
<td>0.89–1.42</td>
<td>0.32</td>
<td>0.47</td>
<td>0.41–0.55</td>
</tr>
</tbody>
</table>

NA, not applicable.
Trends in hypercholesterolaemia prevalence from 2001 to 2009

The prevalence of 5-year-old children with hypercholesterolaemia (TC levels > 5 mmol/l) significantly decreased in girls from 22% in 2001 to 10.8% in 2009 (P<0.001) and in boys from 16.4% in 2001 to 7.9% in 2009 (P<0.001) (Table 2).

In the multivariable model, the interaction between sex and survey year was not statistically significant (P=0.6). As shown in Table 3, OR for hypercholesterolaemia in 5-year-old children were significantly lower in 2009 than in 2001 and in boys.

There was a significant variability across regions (P<0.01). A region in the north-eastern part of Slovenia, bordering Austria, was identified as having significantly higher prevalence of hypercholesterolaemia (Podravska, OR (95% CI): 1.37 (1.18–1.58); P<0.01). Interestingly, a region bordering Italy had a significantly decreased prevalence (Goriška, OR (95% CI): 0.81 (0.67–0.96); P=0.02). Other regions did not differ from the mean.

Discussion

Trends in overweight/obesity prevalence from 2001 to 2009

Slovenia had a total population of 2 million inhabitants and an average number of ~18 000 5-year-old children per generation in the observational period (19). Using nationally representative data, trends in overweight and obesity prevalence in 5-year-old children were determined. The main finding of this study, related to childhood obesity, is that overweight and obesity prevalence (estimated by BMI) in 5-year-olds did not increase. Nevertheless, the prevalence of overweight and obesity is still too high.

Use of IOTF standards in this study has allowed comparison with several other European countries and the USA. The prevalence of overweight and obesity in 5-year-old Slovenian children in 2009 is comparable to European countries like France (~18% prevalence of overweight and obesity in 4-year-old children) (24). The determined prevalence is, however, lower than in other Mediterranean countries such as (neighbouring country) Italy, Greece and Spain and in the British Isles (24).

Stabilisation of the overweight/obesity prevalence in young children is in concordance with very recent findings from several European countries (3). In Germany, the prevalence of overweight and obesity is high, but the trends have stabilised from 2004 to 2008 (4, 25). In England, overweight and obesity in 5- to 10-year-old children have also stabilised recently (26). Prevalence of overweight and obesity has also been stabilised in Sweden, Greece and in The Netherlands (in children of Dutch ethnicity) (27). In addition, our results are also in concordance with the data from the USA where the prevalence of overweight children is not increasing any more since 2000, except in the most obese boys (28). In France, the prevalence of overweight and obesity in 5- to 12-year-old children has even decreased, similarly as in 6- to 13-year-old children in Switzerland (29, 30). It is interesting that while the prevalence of obesity is stabilising this appears to happen on different levels. Different genetic and environmental (cultural) back-grounds possibly contribute to this fact (3).

There is a significant difference in the prevalence of overweight and obesity between 5-year-old boys and girls, being higher in girls. Similar sex-specific difference is observed in the French and Dutch cohorts (27, 31). In our cohort, this gender difference increased over time, as the trend towards stabilisation of overweight/obesity prevalence was more pronounced in boys than in girls. This finding is not in accordance with data from several other countries, where there was a greater stabilisation in the prevalence in girls (3). Again different social status and cultural background can be attributed to this variability (3).

Trends in hypercholesterolaemia prevalence from 2001 to 2009

The main finding of this study is the significant negative trend in the prevalence of hypercholesterolaemia in 5-year-old children from 2001 to 2009. To our knowledge, this is the first study to report such results in pre-pubertal children. In addition, this is the first cross-sectional study where a specific cause was identified that could have influenced the negative trend.

As the influence of the genetic background on TC levels did not change, we speculate that the change in diet (cholesterol intake) of Slovenian 5-year-old children could have influenced the observed trend. Unfortunately, our study did not include the information on participants’ diet. However, in the year 2005, new national nutritional guidelines for the preparation of meals in kindergartens and schools were adopted. Decreased total fat (especially saturated fat) and carbohydrate intake was advocated (18). Decreased dietary total fat intake is associated with decreased TC levels in children (32). According to the
Statistical Office of Republic of Slovenia, over 75% of 5-year-old children visit kindergarten, where they receive up to four meals per day (19). Therefore, the observed decrease in TC level could be at least in part a consequence of changed public health policy.

These results are corroborated by data from the USA where a decrease in TC levels was also shown. They determined decreased total fat, saturated fat and cholesterol intake; specific measures leading to this fact were, however, not identified (33). In addition, the decrease in TC levels is similar in Slovenia and the USA. The TC mean value decreased by 0.27 mmol/l in Slovenian girls and by 0.25 mmol/l in Slovenian boys. In comparison, in 6- to 8-year-old USA children the mean value decreased by 0.13 mmol/l from 1988 to 1994 to 2007 to 2010 (33).

Determination of TC level as a screening tool for dyslipidaemia was made due to the fact that a fasting sample is not necessary in this case. It was previously reported that increased TC levels detect elevated LDL-C levels with 44–50% sensitivity and 90% specificity (34). In order to diagnose dyslipidaemia more than one blood value is needed (35). In Slovenian children with elevated TC levels > 5 mmol/l a fasting lipid profile (TC, LDL, HDL and triglycerides) was carried out consequently. We unfortunately cannot report on these follow-up results, as the identities of screened children were anonymous.

The limitation of our study is that TC levels were not measured in a central laboratory but in laboratories of participating primary care facilities. The methodology, however, was the same, as were the analysers used throughout the study period. An additional limitation of this study is that data on whether the child has attended kindergarten have not been recorded.

Association between BMI and TC levels

The American Academy of Pediatrics recommends screening of children with a family history of premature CVD, a parental history of hypercholesterolaemia or when family history is unknown, and also screening of children with risk factors such as obesity, physical inactivity, hypertension, diabetes mellitus or smoking (35).

Recent studies have now reported that targeted screening of children with a positive family history missed many children with moderate hyperlipidaemia and a substantial number of children with likely genetic dyslipidaemias that would require treatment. They suggest that a universal screening is a better option (15).

In our study, there was no correlation between BMI and TC levels in either girls or boys. Therefore, by screening only overweight/obese children, we would not identify all children with hypercholesterolaemia. These results are in line with the fact that BMI percentiles do not discriminate effectively children with abnormal TC and LDL-C levels, as opposed to the adults (36, 37). Our results, alongside the described data from the literature, therefore further advocate a universal screening for hypercholesterolaemia especially in young children, enabling early prevention of CVD (14, 15).

Conclusions

The trend in the prevalence of overweight/obesity in pre-pubertal 5-year-old Slovenian children has stabilised and there was a significant negative trend in the prevalence of hypercholesterolaemia. We speculate that the changes in public health policies could be the cause of these trends. Interestingly, there was no correlation between BMI and TC levels, further advocating a universal screening for hypercholesterolaemia in children.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References


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