Cortisol concentration affects fat and muscle mass among Polish children aged 6–13 years

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Abstract

Background: Cortisol is a steroid hormone acting as a stress hormone, which is crucial in regulating homeostasis. Previous studies have linked cortisol concentration to body mass and body composition.

Methods: The investigations were carried out in 2016–2017. A total of 176 children aged 6–13 years in primary schools in central Poland were investigated. Three types of measurements were performed: anthropometric (body weight and height, waist and hip circumferences), body composition (fat mass FM (%), muscle mass – MM (%), body cellular mass - BCM (%), total body water - TBW (%)), and cortisol concentration using saliva of the investigated individuals. Information about standard of living, type of feeding after birth, parental education and maternal trauma during pregnancy was obtained with questionnaires.

Results: The results of regression models after removing the environmental factors (parental education, standard of living, type of feeding after birth, and maternal trauma during pregnancy) indicate a statistically significant association between the cortisol concentration and fat mass and muscle mass. The cortisol concentration was negatively associated with FM (%) (Beta=-0.171; p=0.026), explaining 2.32 % of the fat mass variability and positively associated with MM (%) (Beta = 0.192; p=0.012) explaining 3.09 % of the muscle mass variability.

Conclusions: Cortisol concentration affects fat and muscle mass among Polish children.

Trial registration: Cortisol concentration affects fat and muscle mass among Polish children.

Keywords: Body composition, obesity, cortisol level

Background

Cortisol is a steroid hormone produced by the adrenal cortex, widely known as a stress hormone, crucial in the regulation of homeostasis. Cortisol acts as a neuroendocrine mediator of stress responses in organs and effector tissues such as the brain, cardiovascular system, immune system, fat, and muscle tissue [1]. The activation of intracellular glucocorticoid receptors leads to changes in the metabolism, structure, conduction of stimuli in the cell, and changes the expression of genes such as ID2, C3, and OASI [2]. Excess secretion of cortisol is observed in patients with Cushing Syndrome and healthy individuals during stress. Hypercortisolism is dangerous for the body and has an extremely wide spectrum of effects including insulin resistance, obesity, insomnia, hyperglycaemia, elevated cholesterol and triglycerides levels, decreased immune response, and tissue weakening of antioxidant enzyme activity [3].

Numerous studies of the association between body mass/proportion and stress level have been performed...
using questionnaires and measurements of cortisol concentration e.g. [4]. Regardless of the chosen method, the results were inconsistent. Elevated stress levels were observed among individuals with increased BMI [5], although not statistically significant in all cases [6]. Yu et al. (2020) [7] showed that children with obesity displayed significantly lower morning cortisol and higher evening cortisol than normal-weight children. Thus, the diurnal salivary cortisol rhythm is associated with childhood obesity. Dai et al. (2021) [8] showed that lower first-in-morning diurnal and increased night cortisol levels were associated with increased body fat.

Elevated stress levels were found to be associated with decreased body mass among children aged 7–18 years during the political transformation in Poland in the early 1990s [9]. Trauma during pregnancy increased the risk of body weight deficit in 7–10 years-old Polish children, whose prenatal development occurred during the political and economic transformation of 1990s [10].

Most studies of associations between body composition and cortisol concentration have focused on fat and muscle tissue. Cortisol has a catabolic impact on fat tissue, and chronically persistent high concentrations of cortisol affects lipolysis leading to the release of glycerol and free fatty acids. This effect can be a direct action of the hormone or a result of reduced glucose uptake of adipose tissues [11]. High cortisol concentrations affect protein and carbohydrate metabolism in muscle tissue. Elevated cortisol concentration increases the release of gluconeogenesis substrates from peripheral tissues leading to muscle weakness [12]. Physical exercise increases the cortisol concentration due to temporary oxygen stress [13]. Thus, paradoxically elevated concentrations of cortisol can occur among physically active individuals with muscle mass above average.

This study aimed to assess if the cortisol concentration is associated with the composition and proportion of Polish children stratified according to parental education, the standard of living, type of feeding after birth, and maternal trauma during pregnancy.

Materials and methods
The investigations were carried out between December 2016 and December 2017. A total of 176 children in primary schools in central Poland (Lodz, approximately 700,000 citizens) were included in the study. However, due to the lack of questionnaires concerning four children and unsuccessful results of the saliva sample of one child, only results of investigations of 171 children (girls = 93, boys = 78) were included.

The anthropometric measurements of body weight and height, waist and hip circumferences were performed according to Martin’s method [14] by the staff of the Department of Anthropology of the University of Lodz. Using these measurements, body mass index (BMI = body mass (kg)/ body height$^2$ (m$^2$)) and waist to hip ratio (WHR = waist circumference (cm)/hip circumference (cm)) were calculated. The body composition was assessed by the staff of the Biobank Laboratory of the University of Lodz including percent of fat mass - FM (%), the percent of muscle mass – MM (%), percent of body cellular mass - BCM (%), and percent of total body water - TBW (%) using the bioelectrical impedance vector analysis (BIA-101 ASE, Akern, Italy). Information about the standard of living, type of feeding after birth, parental education, and maternal trauma during pregnancy was obtained with questionnaires.

Questionnaire information
The parents of the children provided signed approval for the investigations and filled in questionnaires. The standard of living was divided into three categories, as were also applied in the previous studies [15–17]: (1) low standard of living (‘we live very poorly, insufficient resources for basic needs or we live modestly, we have to be very economical on a daily basis’), (2) medium standard of living (‘we live on average, it is enough for us every day but we have to save on more serious purchases’), (3) high standard of living (‘we live well enough for us without many special savings, or we live very well - we can afford full luxury’).

Children that were breastfed (independently of duration) were included in the breastfed group.

The parental educational background was categorized as: (1) basic (8 years at obligatory primary school and, in some cases, further three years at vocational school), (2) secondary education (4–5 years at secondary school or bachelor degree) and (3) higher education (full university degree).

The information about breastfeeding was categorised as yes (breastfeeding for at least one month) or no.

The children’s mothers reported if they had experienced traumatic events during the pregnancy such as violence in the family, natural disasters, pedestrian injuries and death of family members, and this variable was treated as categories, i.e. yes or no.

Saliva sample collection and preparation
Saliva samples were collected in sterile Falcon tubes (Nest Biotechnology). The children did not eat, drink, chew chewing gum, or brush teeth for the last 30 min before sampling. Cortisol has the highest physiological concentrations in the morning around 8 am and then slightly decreases during the day and evening [18]. The cortisol concentration in serum and saliva is notably higher in the morning (8–9 pm) than in the afternoon (4–5 am) [19]. Due to the diurnal variation in the
cortisol concentration, the cortisol concentration was normalised according to the time of collection and/or sex, according to the equation: \( \frac{n-x}{SD} \), where: \( N \) is the cortisol level, \( X \) is the mean cortisol level for the proper hour (8, 9, 10, 11, 12 pm or 1, 2 am) and/or sex (boy or girl), and \( SD \) – standard deviation for each group.

The normalised cortisol concentrations were divided into three groups: (1) low (below Q1 (lower quartile)), (2) medium (between Q1 and Q3), and (3) high (above Q3 (upper quartile)).

The saliva was collected between 8 am and 2 pm. Immediately after the collection, the samples were stored at 2–8 °C and, on the same day, frozen and stored at −20 °C until the investigation, no longer than three months after collection [20]. Before testing, the samples were thawed and centrifuged at 2 000 g to separate the mucins according to the recommendations of the manufacturer of the ELISA kit (cf. below).

The cortisol concentration of the saliva samples was measured using an enzyme-linked immunosorbent assay (ELISA) kit (DRG® Salivary Cortisol ELISA (SLV-2930), USA).

Each sample was measured in duplicates. The assay included seven standard concentrations. A micro plate reader (SpectraMax i3, Molecular Devices) was used to measure the absorbance at 450 nm. The 4PL method was used to plot the standard curves using an open-source software (https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator/). The concentration range of the investigated samples was in agreement with information supplied by the kit producer. Intra-assay CVs were less than 10 %. Correlation between saliva and serum cortisol level is noticed but the concentration values are not equal, thus it should be considered during drawing the conclusions [21]. However, the study of El-Farhan [22] indicated that measuring the cortisol level in serum, urine and saliva are reliable to each other.

**Statistical analysis**

The children were divided into groups according to the calendar age. The FM (%), MM (%), BCM (%), TBW (%), WHR, and BMI values were standardised according to age and sex. Z-score equivalents were used for the calculations. Additionally, WHO norm BMI for age (2007) was used to arrange the adequate range for underweight, normal weight, overweight and obesity (https://www.fantaproject.org/sites/default/files/resources/FANTA-BMI-charts-Jan2013-ENG_0.pdf). The Chi-squared Pearson test was used to evaluate the sex differences between underweight, normal-weight, overweight, and obese children.

The following variables were normally distributed: FM (%), MM (%), BCM (%), TBW (%), WHR and BMI values, while the cortisol concentration was not normally distributed \((p<0.05)\).

The Mann-Whitney U-test was used to evaluate the differences in cortisol concentration between children with normal and increased BMI.

To assess the differences between cortisol concentration and sex, the standard of living, type of feeding after birth, parental education, and maternal trauma during pregnancy, the Kruskal-Wallis or the Mann-Whitney U-test were used for the analysis of FM (%), MM (%), BCM (%), TBW (%), WHR. The ANOVA or nonparametric Kruskal-Wallis tests were applied to assess possible differences between the cortisol concentration (low, medium, and high) and the composition and proportion of the body. The post hoc Tukey’s HSD test was used to perform multiple comparisons of all pairs of means. Additionally, the Spearman test was applied to calculate the correlation between cortisol concentration and the composition and proportion of the body.

Stepwise forward multiple regression models were used to identify variables that were statistically significantly correlated with FM (%), MM (%), BCM (%), TBW (%), WHR, and BMI. The residues for each body component, BMI, and WHR were used to assess the correlation with the cortisol concentration.

The effect size for each test was performed using online calculator: (https://www.danielsoper.com/statcalc/calculator.aspx?id=48). Cohen’s \( d \) was applied for the Mann-Whitney U test and ANOVA in the case of multiple regression Cohen’s \( \eta^2 \) was done.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures involving research study participants were approved by the Ethical Commission at the University of Lodz (nr 19/KBBN-UL/II/2016). Written informed consent was obtained from all the subjects’ parents.

**Results**

Among all children, 77.19 % had normal BMI (including 43.86 % of girls and 33.33 % of boys), 0.58 % were underweight, the rest of the children with excess BMI 16.37 % were overweight (including 9.94 % of girls and 6.43 % of boys) and 5.85 % were obese (including 0.58 % of girls and 5.26 % of boys) (Table 1).

The cortisol concentration was not statistically significantly different between boys and girls (Table 2).

The cortisol concentration was higher among children from families with a high standard of living (mean=-0.200) than among children with a medium standard of living (mean=-0.354), \( H = 6.532; p = 0.038 \) (Table 3). The cortisol concentration was not statistically
significantly influenced by differences in sex, parental education, maternal trauma during pregnancy, or breastfeeding (Table 3).

We did not find any statistically significant difference in cortisol concentration between children with BMI in norm and excessive BMI in neither of the sexes (Table 4). When the cortisol concentration was divided into three categories: low, medium, and high and compared to the investigated components and proportions of the body, only muscle mass was statistically significant among the categories (F = 3.208; \( p = 0.043 \)). The post hoc Tukey’s HSD test showed that the children with a medium concentration of cortisol had higher muscle mass (mean=-0.060) than those with a high concentration of cortisol (mean=-0.191) (\( p < 0.05 \)) (Table 5). The rest of the tested body composition and proportion components did not show any statistically significantly different results (Supplementary Table 1).

The cortisol concentration was negatively correlated with FM (%) and WHR z-score (R=-0.169, \( p = 0.027 \); R=-0.230, \( p = 0.002 \), respectively) and positively correlated with MM (%) (R = 0.224 \( p = 0.003 \)).

**Stepwise forward multiple regression models**

Multiple regression models were used to assess the correlation between (1) FM (%), BCM (%), MM (%), TBW (%), BMI, and WHR and (2) breastfeeding, maternal trauma during pregnancy, the standard of living, and parental education to evaluate their association with dependent variables and calculate residues for each of dependent variable.

Table 6 shows the statistically significant results of the analyses of the correlations between (1) the composition and proportion of the body and (2) environmental factors among Polish children. The first model explained the correlation between FM (%) and a high standard of living (vs. low) (Beta = 0.709; \( p = 0.033 \)) with the control of standard of living medium (vs. low), higher paternal education (vs. basic), and breastfeeding (vs. no) explaining 3.95 % of the variability of FM (%). MM (%) was positively correlated with higher paternal education (vs. basic) (Beta = 0.618; \( p = 0.025 \)) with the control of secondary paternal education (vs. basic), secondary maternal education (vs. basic), and breastfeeding (vs. no) explaining 6.07 % of the variability. No statistically significant correlation was found between BCM (%) and the investigated parameters. TBW (%) was negatively correlated with a high standard of living (vs. low) (Beta=-0.792; \( p = 0.016 \)) with the control of medium standard of living (vs. low), higher and secondary paternal education (vs. basic), secondary maternal education (vs. basic), and maternal trauma during pregnancy explaining 7.02 % of the variability. BMI was positively

### Table 1

| Sex            | WHO norm - normal weight | WHO norm - overweight | WHO norm - obesity | WHO norm - underweight | Chi^2 Pearson | p  |
|----------------|--------------------------|-----------------------|-------------------|------------------------|---------------|----|
| Girls          | 75                       | 17                    | 1                 | 0                      | 93            | 9.901 | 0.019 |
| % column       | 56.82 %                  | 60.71 %               | 10.00 %           | 0.00 %                 |               |      |
| % row          | 43.86 %                  | 9.94 %                | 0.58 %            | 0.00 %                 | 54.39 %       |      |
| % total among girls | 57                   | 11                    | 9                 | 1                      | 78            |      |
| % column       | 43.18 %                  | 39.29 %               | 90.00 %           | 100.00 %               |               |      |
| % row          | 33.33 %                  | 6.43 %                | 5.26 %            | 0.58 %                 | 45.61 %       |      |
| % total among girls | 132                  | 28                    | 10                | 1                      | 171           |      |

### Table 2

| Variable      | Sex | N   | Mean   | Median | Q1     | Q3     | SD.   | Z     | p     |
|---------------|-----|-----|--------|--------|--------|--------|-------|-------|-------|
| cortisol concentration | Girls | 93  | 1.6935 | 0.9969 | 0.4079 | 2.1771 | 2.7625 | 0.3845 | 0.7006 |
| cortisol concentration | Boys  | 78  | 1.9514 | 0.9361 | 0.4452 | 2.3231 | 2.5542 |       |       |
| cortisol concentration | Total | 171 | 1.8112 | 0.9732 | 0.4175 | 2.2930 | 2.6648 |       |       |
correlated with a high standard of living (vs. low) (Beta = 0.925; \( p = 0.005 \)) and medium standard of living (vs. low) (Beta = 0.675; \( p = 0.041 \)) with the control of higher and secondary maternal education (vs. basic) explaining 5.95% of the variability. The model for WHR was not statistically significantly associated with any parameter. Supplementary Table 2 shows the statistically non-significant results of this analysis.

**Linear regression models using residues**

The obtained residues for each dependent variable in the linear regression models were used to evaluate the correlations between body components and BMI and between WHR and cortisol concentration (Supplementary Table 3). The cortisol concentration was negatively correlated with FM (%) (Beta = -0.171; \( p = 0.026 \)), explaining 2.32% of the fat mass variability, and positively correlated with MM (%) (Beta = 0.192; \( p = 0.012 \)), explaining 3.09% of the muscle mass variability (Table 7). However, in both cases, the effect sizes were very low (0.0238 and 0.0389, respectively).

**Discussion**

The obesity of children is a global problem [23]. Our results showed that 16.37% of the investigated children were overweight (including 9.94% of girls and...
6.43% of boys), and 5.85% were obese (0.58% of girls and 5.26% of boys). The association between cortisol concentration and overweight is unclear. Chu et al. (2017) [24] found increased salivary cortisol concentrations among aged 4–5 years with overweight, and Veldhorst et al. (2014) [25] observed increased hair cortisol concentrations among children aged 8–12 years. Genitsaridi et al. (2018) [6] did not find increased hair cortisol concentration in obese children and adolescents aged 4–18 years. Similar results were obtained by investigations of saliva [26] and plasma among children [27]. Hill et al. (2010) [28] did not observe any association between the cortisol level and fatness among children aged 1–2 years. Our studies showed that children with decreased cortisol concentration had increased fat tissue and decreased muscle tissue. However, the cortisol concentration explained only 2.32% of the fat mass variability and 3.09% of the muscle mass variability, and the effect size was low in each case. Due to the diurnal variation in the cortisol concentration, it is difficult to find the exact value that affects the body composition and proportion. It is still unclear if the elevated cortisol concentration is an effect or cause of obesity. Increased levels of cortisol among obese children may be a consequence of systemic inflammation [29]. In contrary, we did not find any statistically significant difference in the cortisol concentrations between children with

### Table 5: Comparison of the body components/proportions and cortisol concentration in Polish children

| Cortisol concentration | Mean | N  | SD  | Q1   | Median | Q3   | F/H* | p     |
|------------------------|------|----|-----|------|--------|------|------|-------|
| Low                    | -0.134 | 44 | 1.058 | -0.870 | -0.191 | 0.434 | 3.208 | 0.043 |
| Medium                 | -0.070 | 84 | 0.919 | -0.759 | -0.060 | 0.667 |
| High                   | 0.325  | 43 | 0.866 | -0.184 | 0.375  | 0.906 |
| Total                  | 0.012  | 171| 0.956 | -0.707 | -0.040 | 0.727 |

Cortisol concentration: Low: < Q1; Medium: Q1-Q3; High: >Q3
Statistically significant effects (post-hoc Tukey HSD test):
MM z-score:
Low vs. Medium (p < 0.05; Cohen’s d effect size = 0.0646)

### Table 6: A forward stepwise multiple regression model including independent variables explaining body composition: FM (%), MM (%), TBW (%), and BMI (standardised for calendar age and sex) among Polish children

| Dependent variables | Independent variables | Beta  | SE   | t     | p value | Adjusted R² | F    | p value | Cohen’s f² |
|---------------------|-----------------------|-------|------|-------|---------|-------------|------|---------|------------|
| FM (% z-score)      | Breastfeeding - yes (vs. no) | -0.117 | 0.077 | -0.407 | 0.129 | 0.0395 | 2.76 | 0.030  | 0.0411    |
|                     | Paternal education higher (vs. basic) | -0.133 | 0.079 | -0.254 | 0.094 |          |      |         |            |
|                     | Standard of living higher (vs. low) | 0.709 | 0.330 | 2.150 | 0.033 |          |      |         |            |
|                     | Standard of living medium (vs. low) | 0.613 | 0.331 | 1.852 | 0.066 |          |      |         |            |
| MM (% z-score)      | Breastfeeding - yes (vs. no) | 0.359 | 0.269 | 1.334 | 0.184 | 0.0607 | 2.70 | <0.033 | 0.0646    |
|                     | Paternal education higher (vs. basic) | 0.618 | 0.273 | 2.265 | 0.025 |          |      |         |            |
|                     | Paternal education secondary (vs. basic) | 0.464 | 0.281 | 1.654 | 0.100 |          |      |         |            |
|                     | Maternal education secondary (vs. basic) | -0.239 | 0.192 | -1.248 | 0.214 |          |      |         |            |
| TBW (% z-score)     | Breastfeeding - yes (vs. no) | 0.479 | 0.264 | 1.814 | 0.071 | 0.0702 | 2.85 | 0.008  | 0.0755    |
|                     | Paternal education higher (vs. basic) | 0.537 | 0.273 | 1.966 | 0.051 |          |      |         |            |
|                     | Paternal education secondary (vs. basic) | 0.298 | 0.277 | 1.075 | 0.284 |          |      |         |            |
|                     | Maternal education secondary (vs. basic) | 0.172 | 0.070 | 2.46  | 0.0141 |          |      |         |            |
|                     | Standard of living higher (vs. low) | -0.792 | 0.326 | -2.433 | 0.016  |          |      |         |            |
|                     | Standard of living medium (vs. low) | -0.592 | 0.329 | -1.799 | 0.074  |          |      |         |            |
|                     | Maternal trauma during pregnancy - yes (vs. no) | -0.314 | 0.237 | -1.324 | 0.187  |          |      |         |            |
| BMI z-score         | Breastfeeding - yes (vs. no) | -0.326 | 0.261 | -1.252 | 0.212 | 0.0595 | 3.16 | 0.009  | 0.0632    |
|                     | Paternal education higher (vs. basic) | -0.715 | 0.372 | -1.924 | 0.056  |          |      |         |            |
|                     | Paternal education secondary (vs. basic) | -0.488 | 0.385 | -1.265 | 0.208  |          |      |         |            |
|                     | Standard of living higher (vs. low) | 0.925 | 0.323 | 2.864 | 0.005  |          |      |         |            |
|                     | Standard of living medium (vs. low) | 0.675 | 0.327 | 2.063 | 0.041  |          |      |         |            |
normal and excessive BMI. Our previous study of children aged 7–11 years did not show any association between the body components and cortisol level [15]. On the other hand, Yu et al. [7] suggested that the daily amplitude of the cortisol concentration may be more important than the cortisol concentration itself.

We found that the cortisol concentration was increased among children with a high standard of living. These findings might indicate that children from well-off families are under higher pressure, possibly due to increased expectations and increased sports activities [30]. Thus, the elevated cortisol concentrations may be caused by psychological stress and/or increased oxidative stress due to increased physical activity, which may lead to increased muscle mass among children with elevated cortisol concentration.

The parental education level was associated with body composition. Higher and secondary education among fathers was associated with increased muscle mass among the children. Similar results were previously obtained for mothers [31]. Post et al. (2018) [32] found an association between good education of the parents and increased specialization in sports disciplines of the children. Highly educated fathers may pay more attention to the sustainable development of their children and encourage their physical activity, which may lead to increased muscle mass.

**Limitations**

The study has some limitations. Firstly, the sample collection was only conducted once, and it is indicated by some authors that the level of the cortisol collected at the different time points may be different and preferable is the mean value of the a few daily measurements. Additionally, the body composition measurements using the BIA method may give inconsistent results [33, 34]. Thus, validation using DEXA is preferable. Moreover, the traumatic events during pregnancy were measured using a binary scale (yes or no). This method is not precise and may be subjective.

**Conclusions**

We conclude that cortisol concentration is positively associated with muscle mass and negatively associated with fat mass, even when the cortisol concentration was adjusted according to environmental factors. We did not find any statistically significant differences in the cortisol concentration between children with normal BMI and excessive BMI in none of the sexes. The cortisol concentration was increased among children from well-off families.

**Abbreviations**

FM (%): fat mass; MM (%): muscle mass; BCM (%): body cellular mass; TBW (%): total body water; BMI: body mass index

**Supplementary information**

The online version contains supplementary material available at https://doi.org/10.1186/s12887-021-02837-3.

| Table 7 Linear regression models explaining the variability of FM (%) and MM (%) (residues) depending on the cortisol concentration |
|---|---|---|---|---|---|---|---|
| Dependent variables | Independent variables | Beta | SE | t | p value | Adjusted R² | F | p value | Cohen’s f² |
| MM (%) z-score (residuals) | Cortisol concentration stand. | 0.192 | 0.076 | 2.535 | 0.012 | 0.0309 | 6.42 | 0.012 | 0.0389 |

The online version contains supplementary material available at https://doi.org/10.1186/s12887-021-02837-3.

**Additional file 1**

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**Authors’ contributions**

PP-P designed the study, performed laboratory work, analysed the data, prepared the draft and final version of the manuscript and collected the material. EZ and NM designed the study, prepared the manuscript and provided critical comments on the manuscript. AS and IR designed the study and collected the material. DS designed the study and collected the material. MS, MS-K collected the material. The author(s) read and approved the final manuscript.

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**Availability of data and material**

The datasets generated during the current study are not publicly available due to law regulation but are available from the corresponding author on reasonable request.

**Declarations**

**Ethics approval and consent to participate**

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving research study participants were approved by the Ethical Commission at the University of Łódź (nr 19/KBIN-UI/II/2016). Written informed consent was obtained from all subjects’ parents.

**Consent for publication**

Not applicable.

**Competing interest**

The authors declare that they have no competing interests.

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