Improving cardiorespiratory fitness protects against inflammation in children: the IDEFICS study

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EMGG, ASP, CB, WA and LMA, conceptualized and designed the study, collected data, carried out the initial analyses, drafted the initial manuscript and revised the manuscript. VP, DM, WA, TV, MT, GE, SDH and AF designed the data collection instruments, coordinated and supervised data collection and reviewed and the manuscript FL, LI, LL, and HS, designed the study and critically reviewed the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

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Impact:

-Improvements of cardiorespiratory profile during childhood could reverse an unfavourable inflammatory status.

- There is a longitudinal and inverse association between cardiorespiratory fitness and inflammation in children.

-This is the first longitudinal study assessing the relationship between fitness and inflammation during childhood that takes also into account the lifestyle behaviours.

-Results from the present study suggest a protective role of fitness already in childhood.

- Efforts to improve the fitness in children should be aimed as inflammation could trigger future cardiovascular disease

Abstract

Background and aim: Muscular and cardiorespiratory fitness (MF and CRF) have been related to inflammation. Thus, the aim of this study was to assess the relationship between fitness and high sensitivity C-reactive protein (hs-CRP) in European children, both cross-sectional and longitudinally.

Methods: 357 children (46.2% males), aged 2-9 years, with hs-CRP measured, data from muscular and cardiorespiratory fitness, diet quality, objectively physical activity (PA), and screen time, at baseline and follow-up, after 2 years, were included. Z body mass index (zBMI), waist circumference (WC), and fat mass index (FMI) were assessed. MF and CRF were also dichotomized: low-medium quartiles (Q1-Q3) and highest quartile (Q4).

Results: At follow-up, children with the highest CRF (Q4) showed lower probability of having high hs-CRP. In the longitudinal analysis, children who improved their CRF over time, showed a significantly lower probability ($p<0.05$) of being in the highest hs-CRP category at follow-up, independently of the body composition index considered: OR = 0.22 for zBMI, OR= 0.17 for WC, and OR=0.21 for FMI.

Conclusion: Improving CRF during childhood reduces the odds of an inflammatory profile, independently of body compositionand lifestyle behaviours. These highlight the importance of enhancing fitness, especially CRF, to avoid an inflammatory state in children.

Introduction

Inflammation plays a key role in atherosclerosis, from the early changes in the endothelium to progression from fatty streaks to complex plaques (1, 2). C-reactive protein (CRP) is the most widely inflammatory biomarker used in epidemiological studies and it has been associated with cardiovascular risk factors, even in children (3-5).

In adults it has been observed that physical activity is associated with a lower risk of cardiovascular disease (CVD), suggesting that inflammatory biomarkers, like CRP, could make a great contribution to lowered the risk in this association (6).

However, physical fitness seems a more relevant marker of health than physical activity even in youth (7). Fitness has two main health-related components: muscular fitness (MF) and cardiorespiratory fitness (CRF)(7). MF has been associated with cardio metabolic risk (8) and inflammatory markers (9), even after controlling for weight and height, body mass index (BMI), and body fat (10). In epidemiological studies, CRF has been commonly assessed by the 20-m shuttle run test, usually with adaptations for children (11). In previous literature in youth, CRF was found to be inversely related with inflammation, although these findings were not always presented independently of adiposity (12-14). Even in prepubertal children, a study(13) found an inverse association between inflammatory biomarkers and CRF and a positive association with body fat. This highlights the importance of examining the impact of body composition in the association between fitness and inflammation already at young ages.

In addition, there are some lifestyles behaviours that have been related to inflammation in previous literature, even in children, like diet (15), physical activity (16) and sedentary behaviour (17) that need to be considered in the association between fitness and CRP.

To our knowledge, there is a lack of longitudinal studies using standardized and objective measures to understand the association between fitness and inflammation in early life.

Thus, the main aim of this study is to assess, both in cross-sectional and longitudinal design, the association between CRF, MF and high sensitivity CRP (hs-CRP) in a sample of European children, taking into account body composition indices and lifestyle behaviours as covariates.

Material and methods

Study design

The IDEFICS study, Identification and prevention of Dietary- and lifestyle- induced health EFects In Children and Infants, is a multi-centre population-based study, including an intervention component(18), community oriented with a set of intervention modules addressing diet, physical activity and copping with stress. In this sense, two study regions per country were included: a control and an intervention region geographically apart. This study was performed in children from eight European countries: Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain and Sweden. Design and main procedures have been described in detail elsewhere (19). Two main surveys were performed: baseline (T0) between September 2007 and May 2008, and follow-up (T1) between September 2009 and May 2010, after two years.

Authorization from each ethics committee was obtained: Ethics Committee of the Ghent University Hospital (Belgium), National Bioethics Committee (Cyprus), Tallinn Medical Research Ethics Committee (Estonia), Ethics Committee of the University of Bremen (Germany), Medical Research Council of Pécs (Hungary) Ethics Committee of the Local Health Institute in Avellino (Italy), Ethics Committee of Clinical Research of Aragon (CEICA) (Spain), and Regional Ethics Committee of the University of Gothenburg (Sweden). Parents provided written informed consent and children provided oral assent. The study was performed according to the ethical guidelines of the Edinburgh revision of the 1964 Declaration of Helsinki (2000).

Study sample

At baseline, the IDEFICS study included 16,228 children from 2 to 9 years and 11,038 children aged 4 to 11 at follow-up. 4,646 children had data on hs-CRP longitudinally. Among them, children with complete information at both time points, T0 and T1 on several indices and behaviours were included in the current analysis (n=351, 46.4% males). Also, 6 years was the minimal age to perform the 20 m shuttle run test, so the included sample ranged from 6 to 11 years. **Figure 1** summarizes the flow chart of the study population.

Measurements: anthropometric and demographic variables

Anthropometric measurements followed standardized procedures(20). Height was measured with an stadiometer (SECA 225), while weight and percentage of body fat were measured with a child-adapted Tanita BC 420 SMA. Sex- and age-standardized body mass index z-score (zBMI) according to Cole et al. (21) was calculated. Also, FMI was calculated [kg body fat derived from percetange of body fat from the Tanita/ height (m^2)]. WC, as indicator of abdominal fat, was measured using an inelastic tape (SECA 200). The highest parental education level was used as an indicator of socioeconomic status (SES) and was categorized according to the International Standard Classification of Education (ISCED)(22).

Fitness

The upper-body MF was assessed using the handgrip strength test through a dynamometer with an adjustable grip (TKK 5401 Grip D, Takey, Tokyo Japan) (23). Participants were instructed to squeeze continuously for ≥ 2 seconds with the elbow in full extension. The best score of the two attempts for each hand was chosen and averaged. Relative upper-body MF was expressed per kg of body mass (handgrip strength (kg/kg)) (24). The lower-body MF was assessed by the standing long jump test. Participants had to jump as far as forward possible. The distance reached was taken from the take-off line and the heel of the nearest foot at landing. The longest attempt out of 2 was chosen (25).

Based on these two fitness tests, a MF score (MF z-score) was computed by combining upper-body and lower-body results Each of these variables was standardized as follows: z-score = (ith value − mean)/SD. The MF z-score was based on previous studies(24) and calculated as the mean of the 2 standardized scores (handgrip strength and standing long jump).

The CRF level was assessed using the 20 meters' shuttle run test, which estimates the aerobic capacity. The results of all the centres were unified according to the Leger test protocol (26). The number of shuttles was used as an indicator of the cardiorespiratory level with a greater number of shuttles indicating better performance.

Both scores, MF and CRF, were categorized for a better interpretability. The first group included those children in the first, second or third quartile (Q1-Q3); and the group II included those children at the top quartile (Q4). Additionally, combinations of grouping between surveys were created and the cumulative fitness score (MF+CRF), including the MF and CRF as continuous variables, was calculated at T0, T1, and the delta values of this score, i.e. differences between T1 and T0.

Hs-CRP

Children were asked to participate in fasting blood collection, on a voluntary basis (27). The hs-CRP concentrations were measured using latex-enhanced nephelometry (NB2-Nephelometer, Siemens, USA) and the lower limit of detection was 0.02 mg/dL. Children with hs-CRP concentrations higher than 10mg/dL were excluded (28).

Physical activity

Physical activity was objectively measured using an Actigraph uniaxial accelerometer (ActiTrainer or GT1M; ActiGraph, LLC, Pensacola, FL, USA). Children wore the accelerometer for up to seven consecutive days (29). The PA levels were defined according to Evenson cut-offs (30), where we considered moderate-to-vigorous PA (MVPA) minutes per day for the analysis.

Dietary intake

The food frequency questionnaire (FFQ) (31, 32), which was part of the Children's Eating Habits Questionnaire in the IDEFICS study was used to calculate the diet quality index (DQI). As the FFQ used was qualitative, sex-, age-, and country-specific medians of the portion sizes of the corresponding food groups were derived based on the 24-h dietary recall (24-HDR). For the dietary assessment, a computer-assisted 24-HDR, called SACINA ("Self-Administered Children and Infant Nutrition Assessment") was used in T0 and T1 (33).

The DQI was used as a proxy indicator for the overall children's diet. It was developed for preschool children (34), and adapted for children (35) and adolescents (36).

Total screen time

Total screen time derived from the parent-reported questionnaire. The questions included the time spent watching TV, videos and DVDs, and the time using a computer and/ or playing videogames on a weekday and a weekend day separately (37). Finally, the average total screen time in hours per week was calculated.

Statistical analysis

Descriptive study characteristics are shown as mean and standard deviation for continuous variables and number of cases and percentages for categorical variables. Gender differences were obtained by independent samples t-test (continuous data) or chi-square (categorical data). The distribution of hs-CRP was skewed; approximately a third of the sample had values under the detection limit (0.02 mg/dL) in T0 and T1. Thus, subjects were allocated into two categories. The category I included participants under the detection limit and those with values ≤ of the median (≤0.06 mg/dL in males, and ≤0.075 mg/dL in females). The category II included those participants over the median cut-off (>0.06 mg/dL in males, and >0.075 mg/dL in females). Regarding the MVPA recommendations defined by Evenson, the area under curve ≥ 0.85 was used as cut-off.

For the cross-sectional analysis, a multilevel logistic regression analysis (levels: country and intervention versus control area) was performed using the hs-CRP at both time points as dependent variable. This multilevel logistic regression assessed the odds for having a higher inflammatory status when participants were in the highest quartile (Group II, Q4), compared with those who were in the low-medium quartile group (Group I, Q1-Q3), of fitness which were considered as the reference group. The cross-sectional analysis between fitness and hsCRP was performed using three separate models for each body composition indicator (zBMI, WC or FMI) adjusting for potential covariates: sex, age, SES, MVPA, DQI, total screen and levels (country and intervention vs control region).

To analyse the longitudinal association between fitness and hs-CRP several analyses were performed. First, a linear regression was used to assess the association of continuous values of MF, CRF and MF+CRF at T0 and the mean differences over time (T1 – T0) of this cumulative score, with the corresponding hs-CRP levels at T1 as well as the delta values over time of hs-CRP. Several models were performed adjusted for each body composition indicator: zBMI, WC or FMI, at follow-up (T1), and the hs-CRP levels at T0.

Finally, a multilevel logistic regression analysis (levels: country and intervention versus control area) was performed using the hs-CRP at T1 as dependent variable to assess the odds for having a higher inflammatory status when participants presented a specific fitness level (MF or CRF) at baseline and follow-up. For the longitudinal analysis, four categories were created (**Figure 2**): Group I, children being in the low-medium fitness level (Q1-Q3) of MF or CRF at both time points (T0 and T1); Group II, children being in the low-medium fitness level (Q1-Q3) of MF or CRF at T0 and being in the highest quartile at T1; Group III, children being in the highest quartile of the MF or CRF (Q4) at T0, and being in the low-medium fitness level (QI-Q3) at T1; and Group IV, children being in the highest fitness MF or cardiorespiratory fitness (Q4) at both time points (T0&T1). In the analyses, group I was considered as reference.
The longitudinal multilevel logistic regression analysis was applied using two models. Model 1 included separately the three body composition indicators (zBMI, WC and FMI), sex and age at T1, taking into consideration the hs-CRP levels at T0, and it was adjusted by levels (country and intervention vs control region). Model 2 additionally included SES, MVPA, DQI and total screen time, also at follow-up (T1). ç
Sensitivity analysis were applied between included and excluded participants in order to check differences in some of the common measurements. Included participants were older and having

high SES than the excluded ones ($p<0.05$). However, no differences were observed in terms of BMI categories.

The analysis was performed using the Statistical Package for the Social Sciences (version 21.0, SPSS) and Stata (version 13.0) for the multilevel logistic regression. The figures were performed with Excel (Microsoft).

Results

Table 1 shows the main characteristic of the study participants at baseline (T0) and follow-up (T1) by sex. Percentages of children allocated in each group for both MF and CRF, at T0 and T1, are shown in **Figure 3**. Longitudinally, a high proportion of children stayed in the lowest group (Q1-Q3) of the cumulative MF+CRF over time (n= 172, 48%) (data not shown).

OR and 95% CI for the cross-sectional associations between fitness (MF or CRF) and hs-CRP categories are shown on **Table 2**. In T1, children with the highest CRF (Q4) had 65% lower probability of being allocated in the upper category of hs-CRP compared with low CRF, after controlling for body composition indicators (zBMI or WC), and SES, MVPA, DQI and total screen time.

A regression analysis for the longitudinal association of the continuous fitness at baseline, MF and CRF, and the prospective CRP at T1, either as categorical or continuous, was performed. Also, associations between changes in the continuous fitness variables, T1-T0, and prospective CRP, T1, were investigated but no significant associations were found (data not shown).

Finally, **Table 3** shows the OR and 95%CI for the longitudinal associations between the hs-CRP categories, and the established MF and CRF group combinations over time, including three different body composition indices as covariates. Regarding CRF, the strongest associations were found in those allocated in the group which improved their cardiorespiratory fitness level over time (group II), having low-medium CRF (Q1-Q3) at T0 and high CRF (Q4) at T1 in comparison with the reference group, those with low-medium CRF overtime. Specifically, those children allocated in this group II had an 80%, 84% or 80% lower probability of being allocated in the highest hs-CRP category at T1 after controlling for the zBMI, WC or FMI (OR=0.20;

OR=0.16; OR=0.20, p<0.05 respectively) when compared with the reference group, in model 1. Also, in model 2, those children which improved their CRF over time had a 78%, 83% or 79% lower probability of being allocated in the highest hs-CRP category at T1 (OR=0.22; OR=0.17; OR=0.21, p<0.05 respectively) taking into consideration separately the body composition indicators: zBMI, WC or FMI, when compared with the reference group (group I, Q1-Q3 at both time points).

In **supplementary Table (S.1),** mean s-CRP serum at T0 and T1 and delta values (T1-T0) by MF or CRF categories are shown.

Discussion

CRF, was negatively associated with inflammation, assessed by the hs-CRP in European children. These results were found cross-sectionally and longitudinally, controlling for body composition and some lifestyle behaviours. It is important to note that these associations, at least over time, were found independently of several markers of body composition: zBMI, FMI or abdominal fat. In addition, children who improved CRF over time had less odds of having high hs-CRP concentrations.

Cross-sectional analysis

Most of our population were on the low-medium quartiles (Q1-Q3) of MF or CRF over time with more than 60% of subjects in those quartiles for each fitness component and 48% remained in the low-medium quartiles of cumulative fitness, MF + CRF, from T0 to T1. This highlight the necessity of enhancing fitness in childhood which is linked with health, even in children and adolescents (7). Additionally, children with low CRF in early life will also have low levels of fitness years later (38).

At follow-up (T1), those children at the highest quartile of CRF had almost 70% less probability of having a higher inflammatory status in comparison with those with a low-medium level of CRF even when adjusted by zBMI or WC. Previous studies have shown that CRP levels are inversely associated with high levels of fitness even in children (13, 14, 39-41). Out of the two markers of fitness, CRF is the most frequently associated with this inflammatory biomarker (14, 40, 41) and has been associated with a healthier cardiovascular profile in children and adolescents (7).

Longitudinal analysis

Finally, when assessing the longitudinal data, our study found an inverse and significant association on CRF improvement over time and inflammation, independently of several markers of body composition and lifestyle behaviours. Nevertheless, some studies suggest that adiposity is a mediator that could affect the association between CRF and CRP in childhood, as most of the associations were not significant when body fat was entered in the model (12-14), even in pre-pubertal children (13). Even with BMI similar results were found (41, 42). Also in this line, a prospective cohort study from the Australian Schools Health and Fitness Survey concludes that childhood CRF and changes in fitness from childhood to adulthood are inversely associated with adult hs-CRP, and the underlying mechanism through which this occurs is at least partially dependent on adiposity (43). These results from literature suggest that having high levels of CRF may not counteract the negative consequences ascribed to fatness on hs-CRP.

However, there are also studies that suggest a direct link between CRF and hs-CRP, independently of body fat, which is in line with the results of the present study. Data from European adolescents who participated in the HELENA Study, showed that when abdominal adiposity (WC) or weight status (BMI) were included as covariates hs-CRP decreased, but still remained significant (44). It has been already suggested that CRF may have beneficial effects on cardiovascular risk factors independently of fatness in adolescents (40). In children, WC, aerobic fitness, and MF were independently associated with CRP (9). In this study, aerobic fitness was measured in the lab by means of oxygen consumption, which is considered the best physiological measure of aerobic fitness and might provide more accurate values compared with field methods. All together, these findings suggest that a high CRF could imply health-related benefits, independently of body composition. These differences between studies regarding the role of fatness or BMI could be explained by the different ways to measure CRF or the variances on the included populations. In addition, the different body composition indices used in the studies could explain the differences between studies. However, all of these studies emphasized the importance of achieving high CRF levels to have a better hs-CRP profile in childhood.

In the present study, when adding a set of lifestyle behaviours as covariates, such as PA, diet quality and screen time, the association remained significant, highlighting the strong association between CRF and hs-CRP.

It has been shown that PA produces a short-term inflammatory response and a long-term 'anti-inflammatory' effect (45). In children, a previous study found differences in hs-CRP concentrations by categories of self-reported PA (16). However, in the present study, the associations between CRF and hs-CRP were significant, independently of the PA levels. Also, diet has been related to inflammation (46). While dietary patterns have been associated with hs-CRP during childhood also in the IDEFICS sample (15), diet scores are a new tool and have been already associated with health outcomes in children (47). However, we found the associations independently of the DQI. Finally, the link between screen time and inflammation is not well understood; however, it seems to be related with total cholesterol or levels of low-density lipoprotein or with the vascular function (48). Nevertheless, in our sample the differences still remained significant after controlling for body composition indicators.

Some limitations of the present study deserve attention. Firstly, the use of a large set of covariates on the analysis decreased the sample size. However, this also could be considered as a strength adjusting the models for multiple confounders. Secondly, a single blood marker, hs-CRP, was used to determine inflammatory status and may not accurately reflect chronic inflammation. One third of our sample had values of hs-CRP under the detection limit (0.02 mg/dL), as expected for such a young population, for this reason hsCRP had to be categorized.

Also, the use of quartiles to assess level of fitness could be considered a limitation as improvements from Q1 to Q3 over time would be still considered as low fitness level. However, we found no associations when using the continuous fitness variables in the longitudinal analysis. In this sense, our results highlight the importance of high fitness already in childhood even if categorization is not the ideal approach. However, the use of standardized and validated data from eight European countries should be emphasised. Furthermore, the longitudinal design of the analysis gives a better insight of long-term associations. Finally, this is the first study assessing the association between fitness and inflammation in European children that takes into account body composition, objectively measured PA and relevant lifestyle behaviours such as diet quality and screen time.

Conclusion

To sum up, these results suggest that improving CRF over time reduces the odds of having an inflammatory profile in childhood. Additionally, this improvement was independent of PA level, diet quality, screen time and several body composition indices. These results highlight the importance of enhancing fitness during childhood, especially CRF, in order to reduce an inflammatory state and, consequently, the risk of future cardiovascular diseases.

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Figure 1. Flow chart of the population involved in the current study from the IDEFICS study. Abbreviations: T0, at baseline; T1, at follow-up; hs-CRP, high sensitivity C-reactive protein; FFQ, food frequency questionnaire; 24H-DR, 24-hour dietary-recall; zBMI, Body mass index zscore; WC, waist circumference; %Body fat, percentage body fat; FMI, Fat mass index.

Figure 2: Muscular fitness and Cardiovascular fitness grouping design between baseline and follow-up. MF or CRF groups combinations over time: Group I, being in the low-medium MF or CRF quartiles (Q1-Q3) at both time points (T0 and T1), respectively; Group II, being in low-medium MF or CRF quartiles (Q1-Q3) at T0 and being in the highest MF or CRF quartile (Q4) at T1, respectively; Group III, being in the highest MF or CRF quartile (Q4) at T0, and being in the low-medium MF or CRF quartiles (Q1-Q3) atT1, respectively; Group IV, being in the highest MF or CRF quartile (Q4) at both time points, respectively.

Figure 3. Proportion of the study population included in each category of the Muscular or Cardiorespiratory Fitness over time. Abbreviations: MF, muscular fitness; CRF, cardiorespiratory fitness. MF or CRF groups combinations over time: Group I, being in the low-medium MF or CRF quartiles (Q1-Q3) at both time points (T0 and T1), respectively; Group II, being in low-medium MF or CRF quartiles (Q1-Q3) at T0 and being in the highest MF or CRF quartile (Q4) at T1, respectively; Group III, being in the highest MF or CRF quartile (Q4) at T0, and being in the low-medium MF or CRF quartiles (Q1-Q3) atT1, respectively; Group IV, being in the highest MF or CRF quartile (Q4) at both time points, respectively.