Epidemiology of interleukin-6: the 30-year follow-up of the 1982 Pelotas (Brazil) birth cohort study

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\textbf{ABSTRACT}

\textbf{Background:} Cardiovascular diseases are the main cause of death globally. Interleukin-6 (IL-6) is a biomarker of cardiovascular risk.

\textbf{Aim:} To investigate factors associated with IL-6 concentration in serum, from early life up to 30 years of age.

\textbf{Subjects and methods:} In the 2012–2013 follow-up, IL-6 was measured in 2809 participants of the 1982 Pelotas Birth Cohort (1369 males). Multivariable linear regressions, stratified by sex, were performed to evaluate the associations of African ancestry, family income and maternal education at birth, monthly income and education at 30 years, smoking status, harmful alcohol intake, physical activity, and body composition with IL-6, considering a conceptual hierarchical framework.

\textbf{Results:} Males with low educational levels and current smokers had the highest mean IL-6. Among females, African ancestry and low monthly income were associated with the highest mean values for the outcome. Physical activity had an inverse association with IL-6 concentration among females. A direct relationship was observed between the measures of adiposity on IL-6, in both sexes.

\textbf{Conclusion:} Body composition was the main predictor for the outcome evaluated in males and females. Thus, the avoidance of overweight remains an important strategy for the prevention and control of cardiovascular risk and biomarkers associated with these diseases.

\section*{Introduction}

Interleukin-6 (IL-6) is a cytokine with pro- and anti-inflammatory activity (Qu et al. 2014; Schaper and Rose-John 2015). Its pleiotropic function has been described in the immune system, haematopoiesis, regenerative properties, and metabolic regulation (Qu et al. 2014; Schaper and Rose-John 2015). Chronic exposure to IL-6 may promote cardiovascular diseases through metabolic, endothelial, and coagulant mechanisms (Yudkin et al. 2000). In this sense, this cytokine has become an important biomarker of cardiovascular risk (Yudkin et al. 2000; Jansen et al. 2014).

Cardiovascular diseases (CVD) are the main cause of death globally (WHO 2018). In 2017, an estimated 18 million people died of CVD, worldwide. This corresponds to 330 million years of life lost (Roth et al. 2018) and 36.6 million years lived with disability (Kyu et al. 2018). Total deaths from CVD increased by 21.1% in 2017 compared with 2007, despite a decrease in death rates during the period evaluated (Roth et al. 2018). Brazil follows this trend, and CVD is also the main cause of years of life lost, especially among females (Marinho et al. 2018). In 2015, these diseases corresponded to 424,058 (31.2%) of all deaths in the country, and a decrease in death rates by cardiovascular events was observed (Brant et al. 2017).

According to the World Health Organisation (WHO), CVD is determinate by social, behavioural, and metabolic factors (WHO 2018). In this line, efforts also have to be conducted to decrease the prevalence of cardiovascular risk factors, such as prevention actions (WHO 2013, 2018). Higher concentrations of IL-6 were observed in individuals with the presence of metabolic outcomes, including those associated with cardiovascular risk, for instance obesity, as previously described in another birth cohort study, performed in the same city in Brazil as the 1982 Pelotas Birth Cohort (Menezes et al. 2018). Also, studies have found links between socio-economic (Muscatell et al. 2020) and behavioural (Al Rifai et al. 2017; Vella et al. 2017) characteristics and IL-6 concentrations. Although more than 75% of deaths from CVD occur in low and middle-income countries (WHO 2018), the body of evidence about factors associated with IL-6 concentrations is mainly from high-income countries and with specific populations.

Individuals with increased IL-6 serum concentrations presented the highest risk for cardiovascular mortality (Lee et al. 2012; Schnabel et al. 2013). IL-6 signalling occurs via membrane-bound or soluble receptors (Schaper and Rose-John...
Polymorphisms (rs2228145–rs7529229) in the interleukin-6 receptor (IL6R) reduced the signalling in this cytokine, which leads to the attenuation of downstream consequences of IL-6 (Sarwar et al. 2012; Swerdlow et al. 2012). Using the Mendelian randomisation approach, it was reported that these polymorphisms in the IL6R gene reduced the risk of coronary heart disease, evidencing a causal relationship between this cytokine and cardiovascular events (Sarwar et al. 2012; Swerdlow et al. 2012).

Due to the importance of identifying factors associated with IL-6 concentrations for predicting cardiovascular risk and elaboration of prevention actions, as well as to contribute to filling the gaps in the literature about low and middle-income countries, this study aimed to investigate factors associated with serum IL-6 concentration, from early life up to 30 years of age, in the 1982 Birth Cohort from Pelotas (Brazil). Four main factor groups have been tested: (I) ancestry and socioeconomic characteristics at early life; (II) socioeconomic characteristics at adulthood; (III) behavioural variables; and (IV) different measures of body composition at 30 years of age. We hypothesised that adverse socioeconomic and behavioural characteristics that offer cardiovascular risk, and high adiposity measures, are associated with the highest IL-6 concentrations in both sexes in a middle-income country context.

Subjects and methods

The 1982 Pelotas Birth Cohort study was conducted in Pelotas, a city in the extreme south of Brazil, with 214,000 inhabitants that year. From January to December 1982, all three maternity hospitals in the city were visited daily and births were recorded. The original cohort included 5914 live births whose families lived in the urban area of the city. This number represented 99.2% of all births. Participants have been followed up on several occasions. Further details of the study methodology were published previously (Victora and Barros 2006; Barros et al. 2008; Horta et al. 2015). In 2012–2013 (mean age = 30.4 years; SD = 0.35), 3701 participants were evaluated, which, in addition to the 325 known deaths, represented a follow-up rate of 68.1%.

Interleukin-6 concentration

At the 30 years follow-up, non-fasting blood samples were collected from 3453 participants in the Epidemiological Research Centre of the Federal University of Pelotas during the daytime, between 8 am and 8:30 pm. The exclusion criterion for collecting blood samples was pregnancy. All samples were processed in the laboratory and stored in freezers at ultra-low temperature (–80°C). Between July and September 2017, serum IL-6 was measured in duplicate for 2988 participants whose blood samples had been collected in the last follow-up by the Quantikine® HS Human IL-6 immunoassay kit (R&D Systems®, Inc.; Minneapolis, MN55413, USA) and SpectraMax 190 microplate spectrophotometer (Molecular Devices Corp, California, USA). Intra-assay and inter-assay coefficients of variation were 1.9 and 3.4%, respectively.

Out of 2988 participants with IL-6 data at the 30 years follow-up, 137 individuals were excluded who reported continuous use (≥1 month) of hypertension, diabetes, dyslipidaemias, and/or depression medicines. Additionally, 42 participants were not considered because they presented IL-6 serum concentration above 10 pg/mL (Ridker et al. 2000; Amaral et al. 2016), which might be indicative of acute inflammation. Therefore, the final sample comprised 2809 cohort members (n = 1369 males).

Independent variables

The baseline measurements included in the analyses were sex (males/female), family income at birth (in tertiles), and maternal education in complete years (0–4; 5–8; 9–11; ≥12). Genomic ancestry analysis was based on peripheral blood DNA samples from the 3736 cohort members who had been evaluated at 22–23 years of age, using genotype data on 370,539 genome-wide variants to quantify ancestral proportions in each individual. The analyses were carried out as part of the Epigen Initiative as described before (Lima-Costa et al. 2015). African ancestry proportion was categorised in the study as follows: 00–4.59; 4.60–10.99; and 11.00–87.91 (Borges et al. 2017).

The participants’ characteristics at the 30 years follow-up included: monthly income (in tertiles), education in complete years (0–4; 5–8; 9–11; ≥12), smoking status (never smoked; former smoker; current smoker), harmful alcohol intake (Alcohol Use Disorders Identification Test—AUDIT ≥ 8 points) (WHO 2001) and total physical activity (in tertiles) objectively estimated using accelerometers (GENEActiv ActivInsight, Kimbolton, UK), considering a minimum of 10 min of moderate/vigorous physical activity in the mean of minutes/day. The cohort members used the accelerometers on the non-dominant wrist all day and night, including when having a shower and performing other water activities. The period of use varied from four to seven days, including one weekend day. Methodological details of the accelerometry protocol have been described elsewhere (Da Silva et al. 2014). Body composition variables were collected in the 2012–2013 follow-up. Body mass index (BMI) was obtained from weight and height (kg/m²) and the individuals were classified as underweight/healthy weight (BMI < 25.0 kg/m²), overweight (25.0–29.9 kg/m²) and obese (≥30.0 kg/m²) (WHO 2000). Waist circumference (WC) was classified according to the WHO cut-off points as normal (<94 cm for men; <80 cm for women), increased risk for metabolic complications (≥94 and <102 cm for men; ≥80 and <88 cm for women) and significantly increased risk for metabolic complications (≥102 cm for men; ≥88 cm for women) (WHO 2000), measured with a tape measure in the narrowest point of the abdomen. Fat mass was assessed using Dual-energy X-ray absorptiometry (DXA, model Lunar Prodigy Advance-GE®, Germany) (Bielemann et al. 2016). We used the fat mass index (kg/m², categorised in tertiles) estimated by dividing the fat mass (in kg) by square height in metres. The visceral fat thickness and
subcutaneous abdominal fat thickness (centimetres, categorised in tertiles) were measured through abdominal ultrasound imaging, using a 3.5-MHz convex probe interfaced with a Toshiba Xario (Toshiba Medical Systems Corp) ultrasound machine. Details can be found in a previous publication (De França et al. 2017).

**Statistical analyses**

Due to the asymmetric distribution of the IL-6 (pg/mL), we carried out the analyses on the logarithmic scale. Descriptive analyses of the independent variables and outcomes were performed. Variables were included in linear regression models following the conceptual framework adopted (Victora et al. 1997), defined a priori (Figure 1). The distal variables, ancestry, and socioeconomic characteristics at perinatal were incorporated in the first level (African ancestry, family income, and maternal education), followed by socioeconomic variables at 30 years (monthly income and education), in the second level. The hierarchical third level comprised behavioural variables at 30 years old (smoking status, harmful alcohol intake, and total physical activity). The fourth most proximal level was encompassed by estimates of body composition (BMI, WC, fat mass index, visceral fat thickness, and subcutaneous abdominal fat thickness). Each variable of body composition was tested in separate models due to collinearity. Statistical comparisons between categories were performed with tests of heterogeneity or linear trend when there was an indication of a trend. Estimates were adjusted for other variables in the same and distal levels of determination; those that presented $p < 0.20$ at their hierarchical level remained in the model. The normality of residuals and homoscedasticity (homogeneity of variance) were tested graphically. The variance inflation factor was used to evaluate multicollinearity between the explanatory variables. The results were reported in pg/mL for IL-6, in means and confidence interval of 95% after exponential of the logarithm results. The analyses were sex-stratified after a formal interaction test. All statistical analyses were performed using Stata version 14 (StataCorp, College Station, TX, USA).

**Ethical considerations**

In the early phases, verbal informed consent was obtained from the caregivers, according to procedures in Brazil at those times. Recent phases comply with the current requirements of ethical review and include written informed consent from the participants. The 30 years follow-up of the 1982 Pelotas Birth Cohort was approved by the Federal University of Pelotas Ethics Committee, affiliated to the Conselho Nacional de Ética em Pesquisa (National Research Ethics Committee—CONEP)—protocol number: 16/12.

**Results**

The 2809 members of the 1982 Birth Cohort (males $n = 1369$) included in the current analysis corresponded to 75.9% of the individuals interviewed in the 2012–2013 follow-up. Compared with the original cohort, the present study sample was slightly more likely to be female and belong to the intermediate socioeconomic categories at birth. The mothers were at a slightly higher age and lower education level. Also, this sample was less likely to have had low birth weight (Supplemental Table 1). However, the magnitude of these differences was small; the maximum difference was $\sim 3.0\%$ for sex prevalence.

Sex significantly modified ($p$-interaction value $<0.05$) the associations of African ancestry, monthly income, and education at 30 years old, smoking status, and all measures of body composition with the outcome. Thus, the analyses were sex-stratified. The distribution of IL-6 was positively skewed with a median of 1.38 (IQR = 0.97; 2.15) in the total sample, 1.36 pg/mL (IQR = 0.98; 2.04) for males, and 1.42 pg/mL (IQR = 0.97; 2.24) for females. After having been transformed into logarithms, the mean was 0.40 log pg/mL ($SD = 0.60$), 0.38 log pg/mL ($SD = 0.59$), and 0.42 log pg/mL ($SD = 0.61$) for the total sample, males and females, respectively (data not presented).

Table 1 shows the sample description for males. Regarding the characteristics of early life, the most common category for African ancestry proportion was 0.00–4.59% (33.4%), and 44.4% of participants’ mothers had 5–8 years of education. In adulthood, about two in five participants had twelve years or more of education (38.7%). Over 40.0% were current smokers or former smokers and belonged to the upper tertile of physical activity. Harmful alcohol intake was reported for 35.3% of males. The associations of IL-6 and independent variables among men are shown in Table 1. After adjusting IL-6 according to the conceptual framework, participants who had an elevated education showed the lowest mean of IL-6 (1.38 pg/mL; 95% CI 1.30; 1.45) compared

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**Figure 1.** Conceptual framework illustrating the hierarchical relationships between ancestry and socioeconomic characteristics at early life, socioeconomic characteristics at adulthood, behavioural variables at 30 years old, and different measures of body composition with interleukin-6 (IL-6).
with other categories of education. Also, higher means of IL-6 were observed among current smokers (1.57 pg/mL; 95% CI 1.47; 1.69) than in individuals who had never smoked (1.40 pg/mL; 95% CI 1.34; 1.47).

Table 2 describes the characteristics of the sample for females; about one in three participants belonged to the category with the lowest proportion of African ancestry. The maternal education category of 5–8 years had the greatest proportion of female participants (42.7%). At 30 years of age, 46.5% had 12 or more years of education. Concerning behavioural characteristics, ~40.0% were current smokers or former smokers and belonged to the lowest tertile of physical activity. Only 10.7% had harmful alcohol intake. When analysing the associations between IL-6 and the independent variables among women, also shown in Table 2, those within the higher proportion of African ancestry presented higher means of IL-6 (1.64 pg/mL; 95% CI 1.55; 1.74) than those in the categories of the minor (1.48 pg/mL; 95% CI 1.40; 1.57) and intermediate (1.44 pg/mL; 95% CI 1.36; 1.52) proportion of African ancestry. Another socioeconomic variable associated with IL-6 was monthly income in adulthood; females in the first tertile had higher means of IL-6 (1.55 pg/mL; 95% CI 1.48; 1.63), while the means for participants belonging to the second and the third tertiles were respectively 1.51 pg/mL (95% CI 1.43; 1.60) and 1.44 pg/mL (95% CI = 1.33; 1.57). Physical activity was linearly associated with IL-6 among females, with a higher mean for the first tertile (1.63 pg/mL; 95% CI = 1.54; 1.73) followed by the second (1.51 pg/mL; 95% CI = 1.42; 1.60) and third (1.37 pg/mL; 95% CI = 1.28; 1.47) tertiles.

The distribution of body composition variables by sex is presented in Supplemental Table 2. In the adjusted analyses, according to the conceptual framework, the mean of IL-6 increased linearly with the categories of anthropometric variables (BMI and WC) for males and females (Figure 2). The same pattern of dose-response, but with less magnitude, was observed using other different adiposity measures like fat mass index, visceral fat thickness, and subcutaneous abdominal fat thickness (Figure 3).

**Table 1.** Description and mean interleukin-6 concentrations according to independent variables in the 1982 Pelotas Birth Cohort (2012–2013), males (n = 1369).

| Independent variables | Unadjusted | Adjusted |
|-----------------------|------------|----------|
| n (%)                 | p-Value mean | (95% CI) | p-Value mean | (95% CI) | Level<sup>b</sup> |
| African ancestry (%)  | p = 0.18   | 0.13 (1.28; 1.29) | 0.14 (1.28; 1.30) | 1         |
| 0.00–4.59<sup>a</sup> | 448 (33.4) | 1.42 (1.34; 1.50) | 1.41 (1.33; 1.49) | 1         |
| 4.60–10.99            | 445 (33.2) | 1.53 (1.44; 1.61) | 1.52 (1.44; 1.61) | 1         |
| 11.00–87.91           | 447 (33.4) | 1.47 (1.39; 1.55) | 1.48 (1.40; 1.57) | 1         |
| Monthly income (RS)   | p = 0.15   | 0.14 (1.28; 1.29) | 0.14 (1.28; 1.30) | 1         |
| 1st (poorer)          | 432 (31.6) | 1.41 (1.34; 1.49) | 1.42 (1.33; 1.52) | 1         |
| 2nd                   | 491 (35.8) | 1.52 (1.45; 1.60) | 1.53 (1.45; 1.62) | 1         |
| 3rd (richer)<sup>a</sup> | 446 (32.6) | 1.45 (1.37; 1.53) | 1.45 (1.35; 1.55) | 1         |
| Maternal education (complete years) | p = 0.81 | 0.14 (1.28; 1.29) | 0.14 (1.28; 1.30) | 1         |
| 0–4                   | 448 (32.8) | 1.44 (1.36; 1.52) | 1.45 (1.36; 1.54) | 1         |
| 5–8                   | 607 (44.4) | 1.48 (1.42; 1.55) | 1.47 (1.40; 1.55) | 1         |
| 9–11                  | 146 (10.7) | 1.49 (1.35; 1.64) | 1.50 (1.35; 1.67) | 1         |
| ≥12<sup>c</sup>       | 165 (12.1) | 1.44 (1.32; 1.58) | 1.48 (1.33; 1.65) | 1         |
| Smoking status        | p < 0.001<sup>d</sup> | 0.14 (1.28; 1.29) | 0.14 (1.28; 1.30) | 3         |
| Never smoked<sup>a</sup> | 767 (56.4) | 1.40 (1.34; 1.46) | 1.40 (1.34; 1.47) | 3         |
| Former smoker         | 229 (16.9) | 1.47 (1.36; 1.59) | 1.48 (1.36; 1.61) | 3         |
| Current smoker        | 363 (26.7) | 1.60 (1.50; 1.70) | 1.57 (1.47; 1.69) | 3         |
| Harmful alcohol intake (AUDIT) | p = 0.021 | 0.14 (1.28; 1.29) | 0.14 (1.28; 1.30) | 3         |
| No<sup>a</sup>        | 879 (64.7) | 1.42 (1.37; 1.48) | 1.42 (1.36; 1.49) | 3         |
| Yes                   | 479 (35.3) | 1.54 (1.46; 1.62) | 1.53 (1.44; 1.63) | 3         |
| Total physical activity<sup>c</sup> | p = 0.16<sup>d</sup> | 0.14 (1.28; 1.29) | 0.14 (1.28; 1.30) | 3         |
| 1st (0–8.3)           | 284 (20.8) | 1.51 (1.41; 1.61) | 1.53 (1.42; 1.63) | 3         |
| 2nd (8.6–26.83)       | 334 (24.5) | 1.47 (1.38; 1.57) | 1.47 (1.38; 1.57) | 3         |
| 3rd (27.10–53.70)<sup>c</sup> | 443 (31.8) | 1.42 (1.34; 1.50) | 1.41 (1.34; 1.49) | 3         |

AUDIT: alcohol use disorder identification test.

Regressions performed with interleukin-6 on logarithmic scale—results presented in exponential means.

<sup>a</sup>Reference category.

<sup>b</sup>Adjusted for variables in the same and distal levels of determination with p-values < 0.20.

<sup>c</sup>Variable with more missing data (n = 1061).

<sup>d</sup>Linear trend test.
in healthy young adults, are scarce (McDade et al. 2011; Menezes et al. 2019).

In the 30-year follow-up of the 1982 Pelotas (Brazil) Birth Cohort, the main predictor of the outcome was body composition, for both sexes. Concerning the associations of African ancestry, education, monthly income, smoking status, and physical activity with IL-6 concentration, the findings were not consistent between males and females. Sex is a biological variable that distinguishes individuals according to chromosomal karyotype, reproductive organs, and sex hormones (Klein and Flanagan 2016; Buffarini et al. 2020). Gender defines disparities between males and females determined by society or culture (Klein and Flanagan 2016; Buffarini et al. 2020). The immunological response might be influenced by both sex and gender (Klein and Flanagan 2016), thus the biological variable was used to stratify the analyses in the current study. However, gender must also be considered in the interpretation of the results.

A positive association between African ancestry and mean IL-6 serum concentration was observed only among females. Differences in the inheritance of IL-6 genotype in populations of African ancestry may result in higher expressions when compared to populations of European ancestry (Hoffmann et al. 2002). In the observational studies, the findings of ethnic differences in IL-6 serum concentration are heterogeneous (Grunewald et al. 2009; Stowe et al. 2010; Crouch et al. 2020). Although genomic ancestry and ethnicity are distinct, to help fill this knowledge gap, genomic ancestry was included in the conceptual model. The admixed composition of the Brazilian population makes ethnoracial classification complex. Also, it is important to mention that ethnoracial self-reporting in the 1982 Pelotas Birth Cohort was affected by both genomic ancestry and non-biological factors (Lima-Costa et al. 2015).

Socioeconomic factors may be mediators of the association observed between African ancestry and IL-6, like monthly income, a variable that also showed significant association with the outcome only among females in the present study. In Brazil, national studies observed that women with a high proportion of African ancestry are the most disadvantaged population, with a monthly income of less than half of males of European ancestry (IBGE 2019). According to a meta-analysis that included only studies with North American participants, lower socioeconomic status (SES) was associated with higher IL-6 (Z = 0.15; 95% CI = 0.12–0.18) (Muscatell et al. 2020). Longitudinal studies identified higher mean IL-6 among participants with unfavourable socioeconomic conditions (Loucks et al. 2010; Lin et al. 2017).

### Table 2. Description and mean interleukin-6 concentrations according to independent variables in the 1982 Pelotas Birth Cohort (2012–2013), females (n = 1440).

| Independent variables | n (%) | p-Value mean | 95% CI | p-Value mean | 95% CI | Level |
|-----------------------|-------|--------------|-------|--------------|-------|-------|
| **Unadjusted**        |       |              |       |              |       |       |
| **African ancestry (%)** |       |              |       |              |       |       |
| 0.00–4.59*            | 459 (32.8) | 1.47 | (1.39; 1.55) | 1.48 | (1.40; 1.57) | 1 |
| 4.60–10.99            | 470 (33.6) | 1.44 | (1.36; 1.52) | 1.44 | (1.36; 1.52) |       |
| 11.00–87.91           | 469 (33.6) | 1.65 | (1.56; 1.75) | 1.64 | (1.55; 1.74) |       |
| **Family income at birth (terciles)** |       |              |       |              |       |       |
| 1st (poorer)          | 472 (32.8) | 1.64 | (1.55; 1.74) | 1.49 | (1.39; 1.59) | 1 |
| 2nd                   | 532 (36.9) | 1.57 | (1.49; 1.66) | 1.53 | (1.45; 1.62) |       |
| 3rd (richer)*         | 436 (30.3) | 1.52 | (1.44; 1.62) | 1.54 | (1.42; 1.66) |       |
| **Maternal education (complete years)** |       |              |       |              |       |       |
| p = 0.0158            |       |              |       |              |       |       |
| 0–4                   | 495 (34.4) | 1.57 | (1.49; 1.66) | 1.57 | (1.47; 1.67) |       |
| 5–8                   | 614 (42.7) | 1.52 | (1.45; 1.59) | 1.52 | (1.46; 1.59) |       |
| 9–11                  | 154 (10.7) | 1.58 | (1.43; 1.74) | 1.57 | (1.41; 1.74) |       |
| ≥12*                  | 175 (12.2) | 1.33 | (1.22; 1.46) | 1.36 | (1.21; 1.52) |       |
| **Monthly income (R$)** |       |              |       |              |       |       |
| p = 0.002d            |       |              |       |              |       |       |
| 1st (0–700)           | 714 (50.0) | 1.59 | (1.52; 1.66) | 1.55 | (1.48; 1.63) | 2 |
| 2nd (703–1500)        | 442 (30.9) | 1.50 | (1.42; 1.58) | 1.51 | (1.43; 1.60) |       |
| 3rd (1580–12,000)*    | 273 (19.1) | 1.39 | (1.29; 1.49) | 1.44 | (1.33; 1.57) |       |
| **Education (complete years)** |       |              |       |              |       |       |
| p = 0.086d            |       |              |       |              |       |       |
| 0–4                   | 84 (5.9) | 1.69 | (1.49; 1.92) | 1.63 | (1.42; 1.87) | 2 |
| 5–8                   | 259 (18.2) | 1.67 | (1.56; 1.80) | 1.63 | (1.51; 1.77) |       |
| 9–11                  | 418 (29.4) | 1.51 | (1.42; 1.60) | 1.49 | (1.40; 1.58) |       |
| ≥12*                  | 663 (46.5) | 1.45 | (1.38; 1.52) | 1.48 | (1.41; 1.56) |       |
| **Smoking status**    |       |              |       |              |       |       |
| p = 0.31d             |       |              |       |              |       |       |
| Never smoked*         | 848 (59.5) | 1.50 | (1.44; 1.56) | 1.53 | (1.46; 1.60) | 3 |
| Former smoker         | 267 (18.8) | 1.54 | (1.43; 1.65) | 1.54 | (1.42; 1.67) |       |
| Current smoker        | 309 (21.7) | 1.56 | (1.46; 1.67) | 1.49 | (1.38; 1.61) |       |
| **Harmful alcohol intake (AUDIT)** |       |              |       |              |       |       |
| p = 0.18              |       |              |       |              |       |       |
| No*                   | 1272 (89.3) | 1.51 | (1.46; 1.56) | 1.51 | (1.46; 1.57) | 3 |
| Yes                   | 153 (10.7) | 1.62 | (1.47; 1.78) | 1.59 | (1.42; 1.77) |       |
| **Total physical activity** |       |              |       |              |       |       |
| p = 0.004d            |       |              |       |              |       |       |
| 1st (0–8.23)          | 465 (39.6) | 1.60 | (1.52; 1.69) | 1.63 | (1.54; 1.73) | 3 |
| 2nd (8.58–26.57)      | 400 (34.7) | 1.52 | (1.43; 1.61) | 1.51 | (1.42; 1.60) |       |
| 3rd (26.97–125.40)*   | 299 (25.7) | 1.41 | (1.32; 1.51) | 1.37 | (1.28; 1.47) |       |

**AUDIT**: alcohol use disorder identification test.

Regressions performed with interleukin-6 on logarithmic scale—results presented in exponential means.

*Reference category.

*Adjusted for variables in the same and distal levels of determination with p-values < 0.20.

*Variable with more missing data (n = 1164).

*Linear trend test.
Regarding behavioural factors, males who self-reported as current smokers had higher mean IL-6 than those who had never smoked. The biological mechanisms by which smoking impacts inflammation, including an increase in IL-6 levels, are described in the literature (Goncalves et al. 2011). Results of the Multi-Ethnic Study of Atherosclerosis (MESA) showed a positive association between smoking intensity (cigarettes per day) (Al Rifai et al. 2017) and current smoking (McEvoy et al. 2015) on IL-6 concentration. However, no significant difference was observed in mean IL-6 between participants who were never smokers vs. current smokers in the Framingham Heart Study Offspring (Levitzky et al. 2008). The same study reported a positive relationship between pack-years of smoking and IL-6 concentration (Levitzky et al. 2008). Sex did not influence these results (Levitzky et al. 2008; McEvoy et al. 2015; Al Rifai et al. 2017), as opposed to what was observed in the 1982 Pelotas (Brazil) Birth Cohort. We highlight the heterogeneity of the definition of smoking status in the cited references. A review about the intersection of sex, tobacco use, and inflammation reported that the definition of this relationship is complex because many studies do not report whether inflammatory markers differed by sex; the results are not consistent; and there is little knowledge regarding the mechanism by which this interaction occurs, although there is evidence that sex hormones might be involved (Ashare and Wetherill 2018).

The present research observed an inverse relationship between physical activity and the outcome among females. For males, there was no linear trend between the means of IL-6 and physical activity level \( (p = 0.08) \). There are studies with similar results using cross-sectional (Sotos-Prieto et al. 2016; Vella et al. 2017) and longitudinal analysis (Hamer et al. 2012), and reports of null association (Golzarand et al. 2012). The limitations of these references include the fact that physical activity is self-reported, which may be less accurate than objective measures (Vella et al. 2017). In the current study, this variable was objectively measured through accelerometry. Also, most of the studies evaluate the association between physical activity and IL-6 independent of adiposity measures. In this sense, it is important to note that adiposity may be a mediator based on the hypothesis that physical activity modulates IL-6 by reducing adiposity (Ghanemi and St-Amand 2018). Finally, the non-stratification by sex in the references cited makes it difficult to compare the role of sex differences.

Figure 2. Distribution of mean interleukin-6 (IL-6) according to (a) body mass index [males \( (n = 1031) \); females \( (n = 1115) \)] and (b) waist circumference [males \( (n = 1031) \); females \( (n = 1122) \)] in the 1982 Pelotas Birth Cohort (2012–2013). Values are exponential means, with their 95% CI represented by vertical bars. Males adjusted for African ancestry, family income at birth, education at 30 years old, smoking status, harmful alcohol intake, and total physical activity. Females adjusted for African ancestry, maternal education at birth, monthly income and education at 30 years old, and total physical activity. Both \( p \)-values of linear trend test \( <0.001 \).
Although we observed statistically significant differences in mean IL-6 between groups based on some behavioural variables, the differences between groups were small in magnitude. We found mean IL-6 differences above 1 pg/mL only for measures of body composition.

The more proximal level of the outcome included the measures of body composition, more specifically adiposity. The highest mean IL-6 was observed among individuals with the highest adiposity, consistent for all body composition measures, and both sexes. The chronic inflammation linked to obesity confers the proportional relationship between this disease and IL-6 (Eder et al. 2009). Plausible mechanisms include the IL-6 synthesis promoted by adipocytes and macrophages infiltrated in the adipose tissue (Eder et al. 2009).

On the other hand, a meta-analysis showed that a polymorphism in the promoter region of the IL-6 gene (rs1800795) was associated with obesity (Hu et al. 2018). Data from the 1993 Pelotas (Brazil) Birth Cohort, when comparing changes in body composition between the 18 and 22-year-old follow-ups, showed associations in the same direction using BMI, WC, and fat mass percentage as adiposity measurements (Menezes et al. 2018). Furthermore, studies found a positive association between visceral and subcutaneous adipose tissue and this cytokine (Pou et al. 2007; Carroll et al. 2009; Cartier et al. 2009).

Although visceral fat mass had a steeper slope in females, we point out that anthropometric measures (BMI and WC) presented linear relationships with mean IL-6 as well as estimates that were collected using DXA and abdominal ultrasound. This result is relevant for public health because it indicates that anthropometric measures characterised by simple, non-invasive, and cheap collection, present greater comparability with data from the international literature (Brasil et al. 2011) than accurate techniques (Kuriyan 2018) and may be adequate predictors of IL-6 serum and consequently cardiovascular risk.

Some limitations of this study have to be considered. Although it is plausible that socioeconomic, behavioural, and body composition characteristics precede IL-6 serum concentration, due to the cross-sectional design of analyses at

![Figure 3. Distribution of mean interleukin-6 (IL-6) according to (a) fat mass index [males (n = 989); females (n = 1102)]; (b) visceral fat thickness [males (n = 1013); females (n = 1111)]; and (c) subcutaneous fat thickness [males (n = 1021); females (n = 1114)] in the 1982 Pelotas Birth Cohort (2012–2013). Values are exponential means, with their 95% CI represented by vertical bars. Males adjusted for African ancestry, family income at birth, education at 30 years old, smoking status, harmful alcohol intake, and total physical activity. Females adjusted for African ancestry, maternal education at birth, monthly income and education at 30 years old, and total physical activity. All p-values of linear trend test <0.001.
adulthood, it is not possible to determine temporality and consequently causality for these associations. The variables monthly income, education, smoking status, and harmful alcohol intake were self-reported; thus, measurement error and misclassification may occur. Concerning the outcome, IL-6 was measured only at 30 years of age, and caution is needed when interpreting if the IL-6 serum indeed reflected chronic inflammation. However, we excluded participants with IL-6 concentrations above 10 pg/mL, which might be indicative of acute inflammation (Ridker et al. 2000; Amaral et al. 2016), and those taking medications. Also, there is a diurnal variation in IL-6 circulation (Nilsonne et al. 2016), and the analyses did not take into account the time of day of IL-6 measurement, although the blood collections were made during an interval of 12 h. Finally, the results can be generalised for adults with similar characteristics, especially promoting evidence for low and middle-income countries.

Conclusion

Our results promote important insights for public health because they increase the evidence that avoiding obesity and overweight is essential to prevent cardiovascular diseases. The current study highlights a biological mechanism (via IL-6) that, when associated with social and behavioural factors and obesity, might increase cardiovascular risks. Also, most factors associated with IL-6 observed in this study are modifiable and may be the target of intervention actions, considering the sex differences presented in the study.

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Disclosure statement

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