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Consumption of ultra-processed foods and IL-6 in two cohorts from high- and middle-income countries

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Abstract

This study evaluated the association between ultra-processed foods (UPF) on serum IL-6 and to investigate the mediation role of adiposity. Participants were 524 adults from the EPITeen Cohort (Porto, Portugal) and 2888 participants from the 1982 Pelotas Birth Cohort (Pelotas, Brazil). Dietary intake was collected using FFQ when participants were 21 years of age in the EPITeen and 23 years in the Pelotas Cohort. Serum IL-6 and body fat mass were evaluated when participants were 27 and 30 years old in the EPITeen and Pelotas, respectively. Generalised linear models were fitted to test main associations. Mediation of body fat mass was estimated using G-computation. After adjustment for socio-economic and behaviour variables, among females from the EPITeen, the concentration of IL-6 (pg/ml) increased with increasing intake of UPF from 1·31 (95 % CI 0·95, 1·82) in the first UPF quartile to 2·20 (95 % CI 1·60, 3·01) and 2·64 (95 % CI 1·89, 3·69) for the third and fourth UPF quartiles, respectively. A similar result was found among males in the Pelotas Cohort, IL-6 increased from 1·40 (95 % CI 1·32, 1·49) in the first UPF quartile to 1·50 (95 % CI 1·41, 1·59) and 1·59 (95 % CI 1·49, 1·70) in the two highest UPF quartiles. The *P*-value for the linear trend was < 0·01 in both findings. The indirect effect through fat mass was NS. Our findings suggest that the consumption of UPF was associated with an increase in IL-6 concentration; however, this association was not explained by adiposity.

Key words: **Food consumption: Inflammation: IL-6: Adults: Cohort studies**

Ultra-processed foods (UPF) comprise a group within the NOVA (a name, not an acronym) Food Classification System, where the foods are classified according to the extent and purpose of industrial processing[1]. These foods are made with several ingredients, mostly of exclusive industrial use, and are the result of a series of industrial processes[1]. UPF consumption predicts a negative impact on diet quality because UPF are usually energy dense and have high quantities of sugar, Na, saturated and trans fats and low dietary fibre, proteins and vitamins and minerals[2–7]. In the gut microbiome, UPF promote changes that stimulate diverse forms of inflammatory diseases[8] and alterations in the microbiota composition[9]. All of these characteristics may lead to an association between UPF consumption and an increase in inflammation.

Inflammation is a defence mechanism of the organism in reaction to infection and other injuries, intending to restore physiological homoeostasis[10]. Failure in the inflammatory response or continuous exposure to the triggering agent results in chronic inflammation[11]. Chronic inflammation may be classified in high or low grade, with the latter being characterised by minimal or absence of clinical manifestations, and a modest increase in the systemic circulation of inflammatory biomarkers and inflammatory cells[11]. In this sense, IL-6, an inflammatory biomarker, has become a focal point in recent literature as a possible causal factor of cardiovascular events[12,13], which is the main cause of mortality in the world[14].

Previous studies observed a positive association between the Western dietary pattern and serum concentration of IL-6[15–17], with this dietary pattern being characterised by the presence of UPF[8]. On the other hand, findings from a meta-analysis of clinical trials showed that the Mediterranean dietary pattern decreases IL-6 concentrations[18]. Only one study conducted in Brazil evaluated the consumption of UPF and an inflammatory

Abbreviations: IQR, interquartile range; UPF, ultra-processed foods.

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biomarker, in which a cross-sectional positive association was observed between exposure and serum C-reactive protein concentration among females. However, the significance was lost following adjustment for obesity measures[19], suggesting that body composition may be a mediator in this association. Evidence from longitudinal studies showed that UPF consumption is a risk factor for obesity[20–22]. Low-grade chronic inflammation is present in obesity physiopathology[10], and the highest mean of IL-6 has been found among obese individuals[23,24].

Socio-economic characteristics are positively associated with UPF consumption in the 1982 Pelotas (Brazil) Birth Cohort Study[25,26]. In high-income countries, such as Portugal, an inverse association has been observed between intake of UPF and socio-economic factors[27–29]. The comparison of observational studies with different confounding structures is an approach to explore causal inference. If the result is not reproduced in both settings, the relationship may reflect residual confounding[30]. Therefore, consistent results in the two cohorts from Portugal and Brazil may strengthen previous findings. Furthermore, to our knowledge, there is no previous prospective study investigating the relationship between UPF, according to the NOVA Food Classification System, and serum IL-6. Therefore, this study aimed to evaluate the association between UPF consumption and chronic inflammation, measured by IL-6 concentration, and to investigate the mediating role of adiposity, using data from two population-based cohorts from high- and middle-income countries.

Methods

The EPITeen Cohort

The Epidemiological Health Investigation of Teenagers in Porto (EPITeen) is a population-based cohort that recruited adolescents born in 1990 and were enrolled in public and private schools in Porto, Portugal, during 2003–2004. Among the eligible individuals identified, 2159 (77·5 %) agreed to participate. Three years after, at the second follow-up, 1716 (79·4 %) adolescents participated, and a new group of 783 adolescents integrated the cohort for the first time as they moved to the schools in Porto. At the third follow-up (2011–2013), 1764 (60·0 % of the entire cohort) participants were then re-evaluated. The fifth follow-up, which took place in 2017–2018, obtained a follow-up rate of 42·3 % (*n* 1244 participants). Further details on the study methodology have been published elsewhere[31].

In 2017–2018, when participants were 27 years of age, due to budget constrains, 598 participants had IL-6 measurement data. Of these were excluded thirty-two participants who presented an IL-6 concentration above 10 pg/ml because they could be cases of acute inflammation[32,33] and forty-two participants with a total daily energy intake below the first and above the 99th centiles[19,34]. Forty-three participants (7·2 % of the sample) presented values below the detection limit in the IL-6 assay (0·11 pg/ml) and were assigned a value equal to half the detection limit[35]. For the present analysis, the final sample consisted of 524 participants (236 males and 288 females). Comparing with the remaining cohort, the participants included in the analysis were slightly more likely to be females, and there was an underrepresentation of the lowest parental education categories.

The Pelotas cohort

The 1982 Pelotas Birth Cohort is a population-based study conducted in Pelotas, a city in Southern Brazil, with 214 000 inhabitants that year. The original cohort included 5914 live births whose families lived in the urban area of the city (99·2 % of eligible participants). This cohort is described in detail elsewhere[36–38]. For the current analysis, data from the 2004–2005 and 2012–2013 follow-ups were used. Considering known deaths, the follow-up rates were 77·4 % (*n* 4297) and 68·1 % (*n* 3701) when the participants were 23 and 30 years of age, respectively[37,38].

Out of 2988 participants with IL-6 information at 30 years of age, forty-six individuals were excluded due to an IL-6 serum concentration above 10 pg/ml[32,33], and fifty four with a total daily energy intake below the first and above the 99th centiles[19,34]. Therefore, the final sample comprised 2888 cohort members (1402 males and 1486 females). Comparing with the original cohort, the participants included in the analysis were slightly more likely to be females belonging to families in the second tertile of the family income at birth, participants born to mothers aged ≥ 30 years and with 5–8 completed years of education and those with a birth weight ≥ 2500 g were over-represented. A similar distribution was observed for maternal smoking during the pregnancy.

Exposure variable

In the EPITeen Cohort, information on food consumption was recorded using a semi-quantitative FFQ of the previous 12 months applied during the third wave evaluation (2011–2013 at 21 years of age). The previously designed FFQ according to Willett and colleagues[39] was adapted and validated[40,41] for the adult Portuguese population. This instrument comprises eighty-six food or beverage items and a frequency section with nine possible responses ranging from *never* to ≥ 6 *times a day*. Energy and nutrient intake were computed by multiplying the frequency of consumption for each FFQ item by the nutrient content of a standard portion, with intake once a day being equal to one. Seasonal variation of food consumption was also considered according to what participants reported. Energy and nutrient intake were estimated using the Food Processor Plus software (version 7.2, 1997, ESHA Research), based on the USA, Department of Agriculture (USDA). Values for typical Portuguese foods were measured using the Portuguese Tables of Food Composition, typical recipes and data from other studies, as previously described[40,41].

In the Pelotas cohort, when the participants were 23 years old (2004–2005), food consumption of the previous 12 months was evaluated by the FFQ developed and validated by Sichieri and colleagues[42], containing eight-five food or beverage items. The current analysis was performed with data from seventy items with information about the frequency of consumption and the specified portion. To estimate the energy and nutrient intake, the consumption frequency of each item was multiplied by the nutrient content of the specified portion. This computation was conducted with the Brazilian Table of Food Composition (TACO). Alternatively, the USDA values were used for foods

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not found in the Brazilian table. Details of this methodological approach were previously described[25].

In both studies, the items from the FFQ were classified based on the extent and purpose of their processing into four groups according to the NOVA Food System Classification[43,44]. Group 1 comprised unprocessed or minimally processed foods; group 2, processed culinary ingredients; group 3, processed foods and group 4, UPF[43,44]. Supplemental Table 1 shows the classification of all items included in the FFQ according to the NOVA Food System Classification. The percentage of energy from each food group was calculated, relative to the total energy intake of each participant. The main exposure variable considered in this study was the percentage of energy from UPF.

Outcome variable

The majority of blood samples were collected between 08:00 am and 10:00 am following a 12 h overnight fast in the EPITeen Cohort. IL-6 at 27 years was measured by the Luminex technology using the MILLIPLEX® Human High Sensitivity T Cell Panel in the Clinical Pathology Department of the São João Hospital Center, Porto. Females who were pregnant at the time were excluded from the assay. Sensitivity for MILLIPLEX® was 0·11 pg/ml.

In the Pelotas Cohort, non-fasting blood samples were taken from 08:00 am to 08:30 pm. The exclusion criterion for blood samples collecting was a current pregnancy. All samples were processed in the laboratory of the Epidemiological Research Center in the Federal University of Pelotas. Serum concentrations of IL-6 at 30 years were measured in duplicate by the Quantikine® HS Human IL-6 immunoassay kit (R&D Systems®, Inc.) and SpectraMax 190 microplate spectrophotometer (Molecular Devices Corp). Sensitivity for Quantikine HS ELISA from 0·016 to 0·110 pg/ml.

Mediator

The fat mass was assessed using dual-energy X-ray absorptiometry in both cohorts according to standardised procedures[45] when the participants were 27 years old in the EPITeen and 30 years old in the Pelotas Cohort. We calculated the fat mass percentage by dividing fat mass by total body mass.

Confounders

Confounders were evaluated through standard questionnaires in both studies. Data regarding confounders were measured at the 21 and 23 years old in the EPITeen and Pelotas Cohorts, respectively. Two groups of covariates were considered for adjustment analysis:

(i) Socio-economic characteristics. In the EPITeen study, parental education information was considered for the parent with the highest educational level. The number of completed years of formal education was categorised as 'low' (0–9 years of education), 'intermediate' (10–12 years of education) and 'high' (> 12 years of education). For the Pelotas study, self-reported skin colour according to the categories proposed by the Brazilian Institute of Geography and Statistics; monthly income collected in Brazilian reals and education of cohort member classified as 'low' (0–8 years of education), 'intermediate' (9–11 years of education) and 'high' (≥ 12 years of education), considering the highest number of years of education completed.

(ii) Health behaviour characteristics. In both studies, participants provided information regarding current smoking status (no/yes). In the EPITeen Cohort, leisure physical activity was classified as 'low', 'moderate' and 'high' according to answers to a multiple-choice question proposing three subjective intensity categories (mainly sitting, standing/walking and very active)[46]. In the Pelotas Cohort, participants answered the leisure-time domain of the International Physical Activity Questionnaire, long version[47]. Information from International Physical Activity Questionnaire in tertiles was used to classify individuals as 'low' (first tertile), 'moderate' (second tertile) and 'high' (third tertile) level of leisure physical activity.

Due to the non-fasting blood collection, fasting time was included in the adjusted analyses for the Pelotas Cohort. Furthermore, in both cohorts, the analyses were adjusted for energy intake from food sources other than UPF (groups 1, 2 and 3 of the NOVA Food System Classification).

Ethics statement

Ethical approval of the EPITeen Cohort was obtained from the Ethics Committee of the Hospital São João, Porto, Portugal. The follow-ups of the 1982 Pelotas Birth Cohort were approved by the Federal University of Pelotas Ethics Committee, Pelotas, Brazil. Written informed consent was obtained from all participants in both studies.

Statistical analysis

The descriptive analyses were performed using absolute and relative frequencies for categorical variables and as mean and standard deviation (SD) or median and interquartile range (IQR) for continuous variables.

Crude and adjusted linear regression models were used to assess the associations of UPF consumption (exposure), fat mass percentage (mediator) and IL-6 concentration (outcome). The covariables included as potential confounders were consistent with the model previously developed (online Supplementary Fig. 1). The normality of residuals and homoscedasticity (homogeneity of variance) were tested graphically. The variance inflation factor was used to assess collinearity between the potential confounders included in the model.

Direct and indirect effects of UPF consumption on IL-6 concentration were estimated using G-computation[48,49]. The natural direct effect represents the effect of the main exposure on IL-6 concentration that is not captured by the mediator. The natural indirect effect indicates the effect captured by the mediator, fat mass percentage. The total effect is the sum of the natural direct effect and natural indirect effect. In the mediation analysis, socio-economic and health behaviour characteristics were considered as base confounders and energy from food sources other than UPF as post confounder. Fasting time was also analysed as a post confounder in the Pelotas Cohort.

The percentage of energy from UPF categorised into quartiles was used, and supplementary analyses showed the results

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according to UPF consumption as a continuous variable in grams, energy and energy percentage. IL-6 (pg/ml) was assessed continuously and log-transformed due to its asymmetric distribution. Sensitivity analysis was conducted including breads in the UPF group. As a statistically significant interaction by sex was found in the association between UPF consumption and IL-6, data were analysed stratified for males and females in both cohorts. Additionally, data from the two cohorts were analysed separately because a significant interaction between the studies was observed. It was considered statistically significant P-values < 0·05. All analyses were conducted using the Stata statistical software (StataCorp LP), version 14.0.

Results

A total of 524 participants (45·0 % males) of the EPITeen and 2888 (48·5 % males) of the Pelotas Cohorts were included in the current study. Table 1 describes the characteristics of the individuals included in the analyses. In the EPITeen Cohort, more than one-third of the participants' parents had a high educational level. More males smoked and were more physically active than females. The members of the 1982 Pelotas Cohort had predominantly skin colour white. Males were wealthier and more physically active than females. About half of the participants belonged to the intermediate category of education. The proportion of current smokers was similar among males (25·7 %) and females (22·6 %). Females presented a higher fat mass percentage than males in both studies.

Within the EPITeen Cohort, the median of the IL-6 concentration was 2·59 pg/mL (IQR 1·40; 4·10) for males and 2·72 pg/ml (IQR 1·60; 4·43) for females. In the Pelotas Cohort, the median of the IL-6 concentration was 1·36 pg/ml (IQR 0·98; 2·06) for males and 1·44 pg/mL (IQR 0·97; 2·27) for females (Table 1). In the EPITeen Cohort, UPF represented 25·0 % (males) and 24·2 % (females) of the total energy intake. While in the Pelotas Cohort, these products represented 19·2 % and 20·5 % of the total energy intake among males and females, respectively (Table 2). Table 2 also describes the data for daily consumption of UPF in grams, energy and quartiles of UPF. The association among covariates with median IL-6 and consumption of UPF are presented in Supplemental Tables 2 and 3, respectively.

In the adjusted models (Table 3), a positive relationship was observed between UPF consumption and IL-6 concentrations among females in the EPITeen Cohort and males in the Pelotas cohort. EPITeen Cohort females belonging to the third (2·20 pg/ml; 95 % CI 1·60, 3·01) and the fourth (2·64 pg/ml; 95 % CI 1·89, 3·69) quartiles of UPF consumption had a significantly higher mean of IL-6 compared with those in the first quartile (1·31 pg/ml; 95 % CI 0·95, 1·82). In the Pelotas cohort, males within the third (1·50 pg/ml; 95 % CI 1·41, 1·59) and fourth (1·59 pg/ml; 95 % CI 1·49, 1·70) quartiles of UPF consumption had significantly higher means of IL-6 than those in the first quartile (1·40 pg/ml; 95 % CI 1·32, 1·49). Similar results were obtained when using the UPF energy in the EPITeen Cohort and UPF grams in the Pelotas Cohort (online Supplementary Table 4). Findings remained robust in the sensitivity analyses including bread in the UPF category (online Supplemental Table 5).

The association between IL-6 concentration and quartile of UPF consumption, according to fat mass percentage, is presented in Fig. 1. No association between fat mass percentage and IL-6 concentration was found in the EPITeen Cohort. In the Pelotas Cohort, a strong association between fat mass percentage and IL-6 concentrations was observed among males, represented by the three separate lines. Additionally, in the Pelotas Cohort, females in the third tertile of fat mass percentage had a higher mean of IL-6 than those in the first tertile. A significant interaction between UPF consumption and total fat mass percentage on IL-6 concentration was observed for both sexes in the Pelotas Cohort (P-value < 0·001). The relationship between UPF consumption and IL-6 concentration seems to increase according to fat mass percentage among males in Pelotas. In the EPITeen Cohort, there was no significant interaction between the exposure and mediator for males (P-value 0·46) and females (P-value 0·13). The association between UPF and the fat mass percentage is presented in Supplemental Table 6. Among males in the Pelotas Cohort, the consumption of UPF increased as the fat mass percentage increased. No significant association was observed among females in both the Pelotas and EPITeen Cohorts.

Concerning the mediation analysis, Table 4 shows that the fat mass percentage explained 2·7 % (females from the EPITeen Cohort) and 37·5 % (males from the Pelotas Cohort) of the total effect of UPF consumption on IL-6 concentration, although no statistical evidence of an indirect effect was observed. The direct effect was greater than the indirect effect, and it was significant only among females from the EPITeen Cohort.

Discussion

This study included data from two prospective cohorts from Portugal and Brazil. The results showed that an increment in the energy contribution from UPF was associated with a higher IL-6 concentration among females in the EPITeen Cohort and males in the Pelotas Cohort. Furthermore, the non-significant indirect effect in the mediation analysis suggests that the association between UPF consumption and IL-6 was not explained by adiposity.

A relationship between diet and IL-6 concentration was previously described. A meta-analysis of clinical trials indicated that adhesion to the Mediterranean dietary pattern decreases mean IL-6 (-0·42 pg/ml (95 % CI − 0·73, −0·11))[18]. Participants from the British cohort who continued to have a high Alternative Healthy Eating Index score or improved it over a six-year exposure period had a lower IL-6 concentration compared with those who continued to have low Alternative Healthy Eating Index scores. However, this concentration did not change among participants who had a decrease in Alternative Healthy Eating Index scores[50]. This result suggests that the adverse effect of diet on IL-6 concentrations is detectable when the exposure is maintained over a long period[50]. Concerning unhealthy dietary patterns, the opposite has been found. A positive relationship has been suggested between a Western dietary pattern and IL-6 concentrations[15–17].

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Table 1. Characteristics of participants, stratified by sex in the EPITeen and the 1982 pelotas cohorts* (Numbers and percentages; mean values and standard deviations)

	Male (*n* 236)		Female (*n* 288)	
	n	%	*n*	%
EPITeen Cohort				
Socio-economic characteristics at 21 years				
Parental education, *n* (%)				
Low	75	31·8	104	36·1
Intermediate	66	28·0	81	28·1
High	95	40·2	103	35·8
Health-related behaviours at 21 years				
Current smoker, *n* (%)†				
No	145	61·4	199	69·3
Yes	91	38·6	88	30·7
Leisure physical activity, *n* (%)				
Low	85	36·0	103	35·8
Moderate	101	42·8	133	46·1
High	50	21·2	52	18·1
Measurements at 27 years				
Fat mass (%)				
Mean	28·58		39·05	
SD†	6·93		5·80	
IL-6 (pg/ml)				
Median	2·59		2·72	
IQR	1·40–4·10		1·60–4·43	
IL-6 (log pg/ml)				
Mean	0·63		0·65	
SD	1·28		1·36	
Pelotas Cohort	*n* 1402		*n* 1486	
Socio-economic characteristics at perinatal and 23 years				
Skin color				
White	1047	74·6	1107	74·5
Black	221	15·8	250	16·8
Brown/Indigenous/Asian	134	9·6	129	8·7
Monthly income				
1st (poorer)	415	29·6	543	36·5
2nd	486	34·7	499	33·6
3rd (richer)	501	35·7	444	29·9
Education				
Low	538	38·4	443	29·8
Intermediate	695	49·5	777	52·3
High	169	12·1	266	17·9
Health-related behaviours at 23 years				
Current smoker				
No	1042	74·3	1150	77·4
Yes	360	25·7	336	22·6
Leisure physical activity				
Low	393	28·1	959	64·5
Intermediate	372	26·5	296	19·9
High	637	45·4	231	15·6
Measurements at 30 years				
Fat mass (%)				
Mean	24·34		39·10	
SD†	8·82		8·52	
IL-6 (pg/ml)				
Median	1·36		1·44	
IQR	0·98–2·06		0·97–2·27	
IL-6 (log pg/ml)				
Mean	0·39		0·42	
SD	0·59		0·61	

* Values are *n* (%), mean ± SD or median and IQR.
† *n* might not sum to 524 or 2988 because of missing data.

A study, in Brazilian adults, investigated the cross-sectional association between UPF consumption and chronic inflammation measured by C-reactive protein[19]. Among females, a positive association was observed, though it was no longer found after adjusting for BMI. For males, an inverse relationship was found in crude and age-adjusted models. However, it disappeared when adjusting for socio-demographic variables or when the BMI was included in the model[19].

Although a sex difference was observed between the cohorts, our findings were in the same direction. Some hypotheses may

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Table 2. Daily consumption according to the food processing level, by sex in the EPITeen and the 1982 pelotas cohorts (Median values and interquartile range)

	EPITeen Cohort				Pelotas Cohort			
	Males		Females		Males		Females	
Variables	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Grams								
Unprocessed or minimally processed foods	1343·6	1002·3–1715·7	1284·3	1033·2–1648·1	2066·0	1542·8–2878·8	1751·7	1349·6–2315·9
Processed culinary ingredients	18·0	9·5–26·7	15·6	9·0–26·1	48·0	27·4–80·0	48·0	16·0–96·0
Processed foods	231·1	155·4–373·4	135·0	95·8–200·1	301·2	163·7–520·0	161·4	82·9–257·7
Ultra-processed foods	441·7	308·5–651·7	354·1	234·3–515·6	447·3	265·0–690·0	321·6	182·1–526·2
Energy (kcal)								
Unprocessed or minimally processed foods	1125·0	969·8–1428·3	1002·0	846·1–1187·9	2104·7	1533·0–2881·5	1641·5	1216·4–2270·5
Processed culinary ingredients	111·2	60·0–157·8	106·5	59·5–168·1	192·0	109·7–320·0	192·0	64·0–384·0
Processed foods	412·5	297·7–571·9	307·3	219·9–441·1	482·5	300·7–773·1	314·3	164·6–590·5
Ultra-processed foods	586·3	422·8–798·0	474·7	355·7–641·8	702·0	424·4–1107·1	583·4	384·3–904·2
% Energy (% kcal)								
Unprocessed or minimally processed foods	50·7	43·3–57·5	51·5	46·0–58·2	58·7	50·0–66·5	58·5	49·6–67·1
Processed culinary ingredients	4·5	2·6–6·5	5·5	3·1–7·9	5·1	2·4–8·6	5·6	2·0–10·5
Processed foods	17·8	14·2–22·6	15·8	11·7–20·8	13·4	8·4–19·6	11·0	6·2–17·6
Ultra-processed foods	25·0	19·1–32·5	24·2	19·4–30·5	19·2	12·7–26·8	20·5	13·5–28·6
Quartile of ultra-processed foods consumption (% kcal)								
1st	16·0	13·7–17·7	15·8	13·4–17·5	9·2	6·6–11·4	8·7	5·8–11·1
2nd	22·1	20·9–23·3	21·7	20·7–23·2	16·8	15·1–18·4	16·1	14·5–17·9
3rd	27·7	26·1–29·5	27·3	26·4–28·7	23·4	21·6–25·4	23·2	21·5–25·3
4th	36·8	34·5–43·0	35·7	33·7–40·0	33·8	29·8–39·6	34·1	30·4–39·2

Other sources are the sum of the three other food processing groups – unprocessed or minimally processed foods, processed culinary ingredients and processed foods.

be postulated to explain this result. Several food products comprise the UPF group[1]. Soft drinks/sugar-sweetened beverages followed by yogurt and sweet cookies/salty crackers were the UPF mostly consumed by the EPITeen Cohort participants. In the Pelotas Cohort, sweet cookies, soft drinks and chocolate were the three most common UPF. Sweet cookies/salty crackers were more often consumed by females than males in the Portuguese Cohort, while in the Brazilian Cohort, this food product contributed more to the diet of males than females. We highlight that our approach was not designed to focus on a specific food, but to evaluate overall exposure to UPF. Besides, genes, hormones and environmental exposures modulate sex differences on immune responses, which may explain the distinct findings between the cohorts[51].

The effect of the diet on the gut microbiome homoeostasis has been largely recognized[52]. Several compounds and characteristics of the Western dietary pattern, marked by high consumption of UPF, may create deleterious changes in the microbiome environment and lead to oxidative status, inflammation and secretion of inflammatory biomarkers, such as the IL-6[8,52,53]. Although the body of evidence is mainly from animal studies, and about isolated components of UPF[8], a recent study conducted in a Spanish population indicated that consumption higher than five servings per day of UPF may promote changes in the microbiota composition differently in women and men[9].

Also concerning the contrast between the two studies, it is established that socio-economic characteristics influence UPF consumption. In high-income countries, the consumption of these products is inversely associated with the socio-economic position[27–29], while the opposite has been found in middle-income countries[54–56]. Our analyses did not find an association between exposure and parental education in the EPITeen Cohort. In the 1982 Pelotas Birth Cohort, UPF consumption was greater among participants with the highest educational level and income and females who self-reported white skin colour (online Supplemental Table 3). Additional analysis was carried out including the income and education of the participant in the EPITeen Cohort model. Even with a reduction in the sample size, the results remained robust, after adjusting for three socio-economic variables, health behaviour characteristics and energy intake from food sources other than UPF, females in the third and fourth UPF consumption quartiles presented higher mean IL-6 than those in the first. Among males, no statistically significant results were observed.

Regarding the mediation analysis, the role of adiposity may have been more powerful among Brazilian males than in Portuguese females because we observed a strong association between fat mass and IL-6 concentration among males from the 1982 Pelotas Birth Cohort, although our findings did not suggest a significant indirect effect through body fat mass. Moreover, the results indicated that the association between UPF consumption and serum IL-6 seems to be explained by a possible direct mechanism. A plausible hypothesis is based on the link between diet and oxidative stress[10,57]. The negative impact of UPF consumption on the dietary nutrient profile has been reported[58], including evaluations in Portuguese[6] and Brazilian[2,7] populations. Concerning high energy intake associated with UPF consumption[43], it is well established that dietary energy restriction may decrease inflammation[10]. In this sense, as total energy intake was corrected using the percentage energy contribution from UPF in our approach[19], we consider it improbable that total energy intake has interfered in the findings. Another assumption is based on a recent positive association found between UPF consumption and urinary

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Table 3. Unadjusted and adjusted linear regression coefficients between consumption of ultra-processed foods (% of total energy) and IL-6, by sex in the EPITeen and the 1982 pelotas cohorts (Mean values and 95 % confidence intervals)

	IL-6 (pg/ml)																
	EPITeen Cohort									Pelotas Cohort							
Quartile of consumption of UPF	1st*		2nd		3rd		4th			1st*		2nd		3rd		4th		
	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	*P*-value†	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	*P*-value†
Males																		
Crude	2·21	1·60, 3·06	1·63	1·16, 2·30	1·85	1·33, 2·58	1·83	1·33, 2·51	0·53	1·43	1·35, 1·52	1·43	1·34, 1·52	1·49	1·40, 1·58	1·56	1·46, 1·67	0·03
Model 1	2·22	1·61, 3·07	1·68	1·19, 2·37	1·80	1·30, 2·51	1·82	1·32, 2·50	0·46	1·40	1·32, 1·48	1·42	1·34, 1·51	1·50	1·41, 1·59	1·60	1·50, 1·70	< 0·01
Model 2	2·18	1·57, 3·02	1·66	1·18, 2·34	1·80	1·29, 2·50	1·88	1·36, 2·61	0·62	1·40	1·32, 1·49	1·42	1·34, 1·51	1·50	1·41, 1·59	1·59	1·49, 1·70	< 0·01
Females																		
Crude	1·35	0·99, 1·86	1·78	1·31, 2·41	2·20	1·61, 3·01	2·51	1·82, 3·47	< 0·01	1·65	1·55, 1·76	1·52	1·43, 1·62	1·46	1·37, 1·55	1·50	1·41, 1·59	0·03
Model 1	1·36	0·98, 1·88	1·77	1·30, 2·40	2·18	1·59, 2·99	2·54	1·83, 3·54	< 0·01	1·59	1·49, 1·70	1·52	1·42, 1·61	1·47	1·38, 1·57	1·53	1·44, 1·62	0·36
Model 2	1·31	0·95, 1·82	1·76	1·29, 2·39	2·20	1·60, 3·01	2·64	1·89, 3·69	< 0·01	1·60	1·49, 1·71	1·52	1·43, 1·62	1·47	1·39, 1·57	1·52	1·43, 1·62	0·30

UPF, ultra-processed foods.
Regressions performed with IL-6 on logarithmic scale – results presented in exponential means.
Model 1: adjusted for socio-economic and health-related behaviour characteristics (EPITeen: parental education, smoking status and leisure physical activity; Pelotas: skin color, monthly income, education, smoking status, leisure physical activity and fasting time).
Model 2: adjusted as in model 1 plus energy intake from food sources other than ultra-processed.
* Reference category.
† *P*-values for the linear trend.

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Table 4. Estimated direct and indirect effects of ultra-processed food consumption (% of total energy) on IL-6 mediated through fat mass percentage in the EPITeen and the 1982 pelotas cohorts
(Mean values and 95 % confidence intervals)

	Total effect		Natural direct effect		Natural indirect effect	
	OR	95 % CI	OR	95 % CI	OR	95 % CI*
EPITeen Cohort						
Males	–0·037	–0·186, 0·111	–0·017	–0·177, 0·142	–0·020	–0·058, 0·018
Females	0·232	0·083, 0·380†	0·225	0·076, 0·374†	0·006	–0·012, 0·024
Pelotas Cohort						
Males	0·038	0·009, 0·068†	0·024	–0·007, 0·055	0·015	–0·002, 0·031
Females	–0·016	–0·046, 0·013	–0·010	–0·043, 0·023	–0·007	–0·030, 0·016

* Mediated by fat mass percentage.
† *P*-value < 0·05.

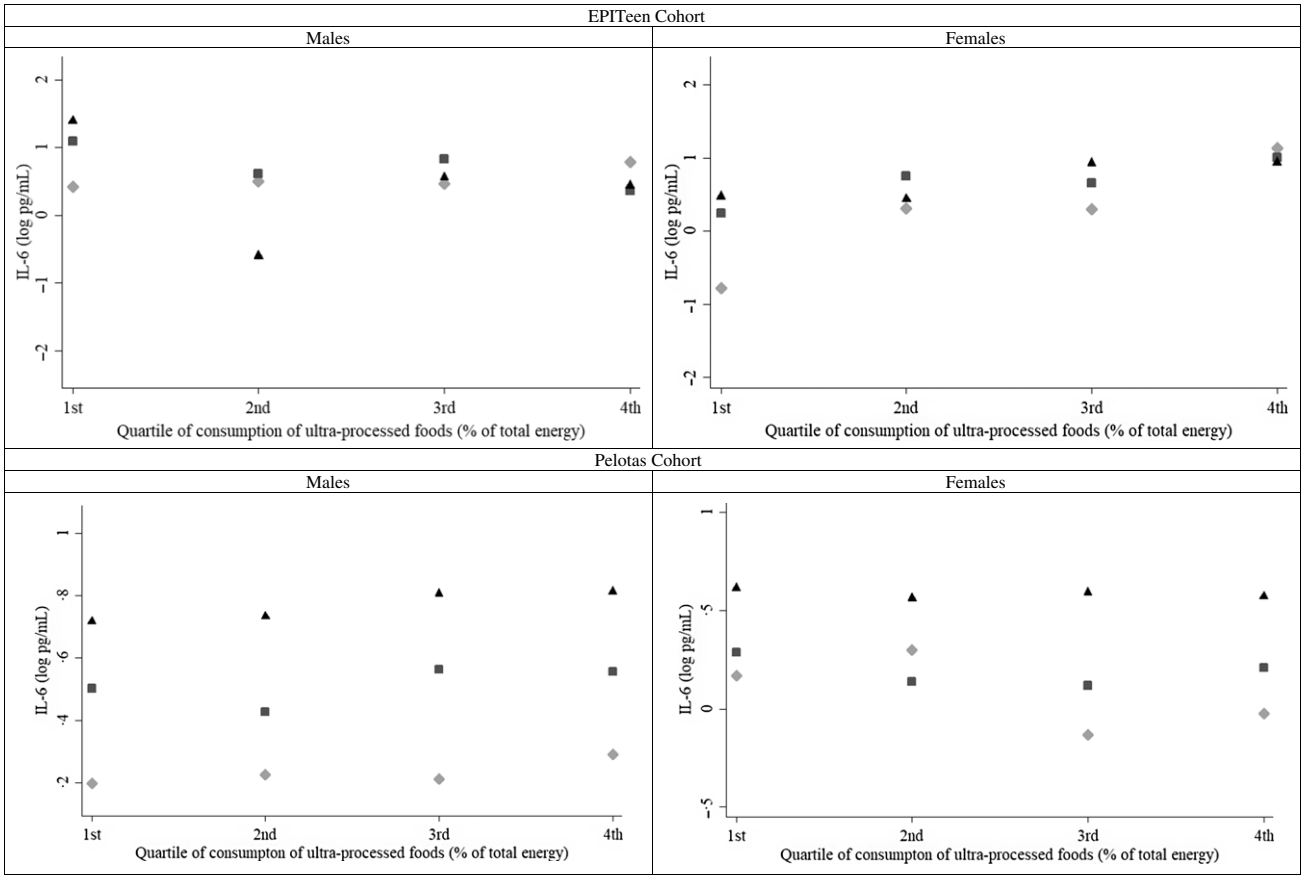

Fig. 1. Adjusted means of log IL-6 according to the consumption of ultra-processed foods and fat mass percentage. Estimates are adjusted for socio-economic and health-related behaviour characteristics (EPITeen: parental education, smoking status, leisure physical activity and energy intake from food sources other than ultra-processed; Pelotas: skin color, monthly income, education, smoking status, leisure physical activity, fasting time and energy intake from food sources other than ultra-processed). Fat mass (%), ◆, 1st; ■, 2nd, ▲, 3rd

metabolites phthalate[59], substances commonly present in packaged foods. These food contaminants may increase the serum concentrations of inflammation and oxidative stress markers[60,61]. Similarly, the consumption of unprocessed or minimally processed foods was inversely related to urinary phthalate metabolites[59], and these foods have antioxidants that may prevent the syntheses of reactive molecules[10]. Furthermore, alterations promoted by UPF in the gut microbiome inducing inflammation[8] could be postulated as a possible indirect mechanism that was not investigated in the current study.

Strengths and limitations of this study

The main strengths of this study are its prospective design, along with an evaluation of data from two settings with distinct socio-economic status, and results in the same direction, despite the observed differences between males and females. EPITeen

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and Pelotas Cohorts were population-based samples, supporting the external validity of our results. The predominant body of evidence about UPF and health outcomes has defined the exposure as the percentage of energy from UPF, the same approach used in this study. We also presented results according to UPF in grams and energies (online Supplemental Table 4) to allow for future comparisons in the literature. Additionally, as it is not clear whether bread should be defined as UPF or not[1], a sensitivity analysis was conducted for this specific food group. Moreover, the fat mass percentage was estimated using an accurate method of body composition assessment[45].

Nevertheless, several potential limitations must be considered. Regarding the assessment of dietary intake, the FFQ used in both cohorts were not specifically developed to apply the NOVA Food Classification System. Therefore, non-differential classification error cannot be ruled out, potentially leading to an underestimation of the magnitude of the associations found[62]. Also, we highlight that in the current study, the energy contribution of UPF accounts for a maximum of 25·0 %, similar to the results found in representative samples of Portuguese[6] and Brazilian[63] populations that evaluated dietary intake through 24-h dietary recall questionnaires. As the number of food items in the FFQ of the EPITeen and Pelotas Cohorts was different, to improve the comparability between the two studies, we ran analysis adjusted for energy intake from other NOVA Food Classification System groups[64].

Dietary information was collected about six and seven years before the outcome data in the Portuguese and Brazilian cohorts, respectively. Therefore, changes in diet that possibly occurred during the follow-up should be considered in the interpretation of the findings' strength. Comparing the dietary data from the 1982 Pelotas Cohort is possible to identify a slight increase in the proportion of total energy from UPF between 23 and 30 years of age[26]. Through observation of national data, a similar tendency may have occurred in the Portuguese cohort[6]. According to mentioned above, the adverse effect due to an increase in UPF consumption during the follow-ups of the cohorts in the current study may not influence the IL-6 concentration[50]. In addition, the absence of repeat measurements of dietary patterns would be more likely to lead to underestimation of the associations[65]. In this sense, the unique assessment of dietary intake seems to follow the assumptions to evaluate the long-term effects of diet in longitudinal analysis[66].

Concerning the outcome data, the lack of repeat data on IL-6 concentration suggests caution when interpreting whether IL-6 serum indeed reflected chronic inflammation, thus possible cases of acute inflammation were excluded. Although differences in assay methods to measure IL-6 serum concentration between the EPITeen and Pelotas Cohorts did not affect the effect measure, it could explain the higher absolute concentration of IL-6 among the EPITeen Cohort participants. A study that compared results produced by R&D Systems ELISA and multiplex immunoassay found significantly greater IL-6 concentration using the second method[67]. Unfortunately, we do not have information concerning the period of the women's menstrual cycle when blood collection was performed, and it can impact the food choices and IL-6 levels. However, as the sample sizes are large and due to the variability of the menstrual cycle, this limitation could not bias the results.

Comparison between participants non-included and included in the analysis showed that attrition bias cannot be ruled out. However, the magnitude of differences was minimal suggesting that could be likely due to the large sample size and not to systematic differences between participants. Finally, considering the multiple factors that can affect the measured associations and the observational design of the current study, the residual confounding could be a potential limitation.

In conclusion, this study observed an association between UPF consumption and IL-6 serum concentration. The mediation analysis did not find a significant effect for adiposity. Our results support a possible link between UPF consumption and inflammation. The findings need to be confirmed in larger prospective cohorts in other settings, mainly aiming to understand the sex differences and using at least two inflammatory biomarker measurements. Nevertheless, recommendations for decreasing the UPF intake and promoting unprocessed or minimally processed foods in the diet may contribute to mitigate chronic inflammation, and hence cardiometabolic disorders.

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The authors' responsibilities were as follows: F. S. S., I. O. O., G. C. M., C. L. and D. P. G. designed the research; F. S. S.

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performed the data analysis and wrote the first draft of the paper; E. R. and C. L. coordinated and supervised data collection of the EPITeen Cohort; B. L. H., and D. P. G. coordinated and supervised data collection of the 1982 Pelotas Birth Cohort. All authors critically reviewed and approved the final manuscript.

There are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114522000551

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