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Dietary exposure to food contaminants and their effects on metabolic outcomes from childhood to adolescence

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TESE DE DOUTORAMENTO EM SAÚDE PÚBLICA APRESENTADA
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Sofia Almeida Costa

Dietary exposure to food contaminants and their effects on metabolic outcomes from childhood to adolescence

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“Todas as pessoas crescidas já foram um dia crianças
(mas poucas se lembram disso).”
Antoine de Saint-Exupéry

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Paper I [Published]

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Paper V [Submitted]

Costa SA, Severo M, Silva CC, Lopes C, Torres D. Associations between food contaminants exposure and pubertal development at 10 and 13 years old: findings from Generation XXI cohort.

I, at this moment, declare that this dissertation is my work result and my entire responsibility. I actively contributed to the definition and operationalisation of the hypothesis tested in the papers above described, as well as to the data analysis and interpretation. I wrote the entire draft of all manuscripts included in this thesis and actively prepared their final versions. I collected data in the 13-year follow-

up of the Generation XXI birth cohort, enrolling the trained Interviewers team. I handled data and checked and cleaned dietary intake databases, both for datasets from Generation XXI and the National Food, Nutrition and Physical Activity Survey – IAN-AF 2015-2016. I also contributed to the statistical analysis and the writing of an e-book and a leaflet (as second author) targeted at the general population and health professionals, with scientific evidence related to food contaminants.

Within the scope of a Doctoral Program in Public Health at the Faculty of Medicine of the University of Porto, and in EPIUnit – Epidemiology Research Unit – Institute of Public Health of the University of Porto and the Laboratory for Integrative and Translational Research in Population Health (ITR), this thesis was developed under the supervision of Professor Duarte Torres (University of Porto – Faculty of Nutrition and Food Sciences) and co-supervision of Professor Carla Lopes (University of Porto – Faculty of Medicine and Institute of Public Health).

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- The Generation XXI cohort, funded by Programa Operacional de Saúde – Saúde XXI, Quadro Comunitário de Apoio III and Administração Regional de Saúde Norte (Regional Department of Ministry of Health).
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LIST OF ABBREVIATIONS

Abbreviations

ADI	Acceptable Daily Intake
BMD	Benchmark Dose
BMDL	Lower confidence limit of the Benchmark Dose
$BMDL_x$	Lower confidence limit of the Benchmark Dose for a x% extra risk
$BMDL_{10}$	Lower confidence limit of the Benchmark Dose for a 10% extra risk
BMI	Body Mass Index
BPA	Bisphenol A
bw	Body Weight
DBP	Diastolic Blood Pressure
DNA	Deoxyribonucleic acid
EC	European Commission
EFSA	European Food Safety Authority
EPIC	European Prospective Investigation into Cancer and Nutrition
FCM	Food Contact Materials
GXXI	Generation XXI birth cohort
HBM4EU	Human BioMonitoring for the European Union
IAN-AF 2015-2016	National Food, Nutrition and Physical Activity Survey 2015-2016
LOD	Limit Of Detection
LOQ	Limit Of Quantification
NHANES	National Health and Nutrition Examination Surveys
NOAEL	No-Observed-Adverse-Effect Level
SBP	Systolic Blood Pressure
t-TDI	Temporary Tolerable Daily Intake

TDI	Tolerable Daily Intake
TWI	Tolerable Weekly Intake
WC	Waist Circunference
WHO	World Health Organization
z-BMI	Body Mass Index z-score
z-DBP	Diastolic Blood Pressure z-score
z-SBP	Systolic Blood Pressure z-score
%FM	Percentage (%) of Fat Mass

ABSTRACT

Background

Diet plays an essential role in human health, providing energy and crucial nutrients for the adequate growth, development and maintenance of the body's homeostasis. However, the simple idea that energy imbalance is the leading cause of the escalating of obesity and other related comorbidities has been increasingly placed into question. Previous studies assessed the effect of exposure to unconventional risk factors on health, such as food contaminants. Acrylamide and bisphenol A (BPA) are food contaminants formed during thermal processing or migrating from food contact materials, respectively, which may negatively affect human health. Children and adolescents are a vulnerable age group due to the relatively higher food intake per body weight, and incomplete maturation of metabolic systems or key organs.

Acrylamide has the ability to form adducts with proteins and nucleic acids, causing genotoxicity, carcinogenicity, neurotoxicity, hepatoxicity, cardiovascular toxicity and reproductive toxicity. Recent studies suggested that acrylamide is a potential endocrine disruptor and may affect cardiometabolic biomarkers and puberty development through mechanisms related to oxidative stress, lipid peroxidation, adipogenesis, and hormonal disruption.

BPA may interfere with oestrogen receptors and, consequently, with the endocrine system, allowing BPA to be classified as an endocrine disruptor. Moreover, chronic BPA exposure, even at lower concentrations, may impair human health, leading to the development of metabolic diseases and pubertal development disarrangements.

Epidemiological longitudinal studies evaluating the association of these food contaminants exposure in metabolic outcomes from childhood to adolescence are limited, and the uncertainties remain to be clarified.

Objectives

The main objective of the present thesis was to assess the effects of food contaminants exposure, particularly acrylamide and bisphenol A, on the development of metabolic outcomes in children and adolescents' health. Thus, the specific objectives to be answered were:

1. To assess dietary exposure to xenobiotic compounds derived from food processing and packaging materials (acrylamide and bisphenol A), applying direct and indirect methods:

1.1. To assess dietary exposure to acrylamide by applying self-reported dietary assessment methods;

1.2. To assess dietary exposure to bisphenol A by applying self-reported dietary assessment methods and urinary metabolites;

2. To longitudinally assess the xenobiotic compounds exposure and to test the association of food contaminants exposure with health outcomes from childhood to adolescence:

2.1. To estimate the risk assessment of food contaminants, namely acrylamide and bisphenol A;

2.2. To longitudinally assess the association between xenobiotic compounds exposure (acrylamide and bisphenol A) and cardiometabolic outcomes;

2.3. To assess the association of acrylamide and bisphenol A exposure on pubertal development at 10 and 13 years old.

Methods

This thesis incorporates data from the Generation XXI (GXXI) birth cohort (Porto, Portugal, 2005-2006), as well as from the Portuguese National Food, Nutrition, and Physical Activity Survey (IAN-AF 2015-2016).

The Generation XXI birth cohort enrolled at baseline 8647 newborns (gestational age >24 weeks) from five public maternities in the Porto Metropolitan area. Follow-up evaluations were conducted at ages of 4 (2009–2011, 86% participation), 7 (2012–2014, 80% participation), 10 (2015–2017, 76% participation), and 13 (2018–2020, 54% participation, interrupted due to COVID-19 pandemic). During each evaluation, trained researchers conducted face-to-face interviews using structured questionnaires, while trained health professionals collected anthropometric measurements (weight, height, waist circumferences), body composition data (bioimpedance), blood pressure, blood samples (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, glucose, insulin) and urine samples (BPA concentration).

Fat mass percentage (%FM) was estimated, and age and sex-specific body mass index z-score (z-BMI), the homeostatic model assessment for insulin resistance (HOMA-IR), and age-, sex- and height-specific for systolic and diastolic blood pressure z-scores (z-SBP, z-DBP) were computed.

Pubertal development was assessed by trained health professionals at the ages of 10 and 13 using the Tanner scale.

Dietary information was collected through three-day food diaries (two weekdays and one weekend day), computerised and categorised using the FoodEx2 classification system. The participant's main caregiver filled the three-day food diary at 4, 7, and 10 years, whereas at the 13-year follow-up, the food diary was filled by the adolescent with the help of parents/caregivers.

In the IAN-AF survey, a total of 5811 participants aged 3 months to 85 years completed two interviews. Among participants under 18 years, 1959 provided complete data from two-days dietary interviews. Trained nutritionists collected additional information on different dimensions, namely sociodemographic characteristics, general health data, the practice of leisure time physical activity, sedentary behaviours, and anthropometrics.

Dietary intake was obtained through two non-consecutive one-day food diaries for children under 10 or 24-hour recalls for the remaining participants. This information was computerised using the eAT24 software, a validated tool integrating the FoodEx2 classification system.

Results

A probabilistic methodology was used to estimate the daily dietary exposure to acrylamide. Three possible values representing the distribution of acrylamide occurrence in foodstuffs were randomly attributed to each food item, accounting for intra and inter-individual variability. Portuguese children aged 1 to 2 years old presented the higher median of dietary exposure, 0.75 µg/kg bw/day (95th percentile: 1.41 µg/kg bw/day), followed by children of 3 to 9 years of age with 0.69 µg/kg bw/day (95th percentile: 1.32 µg/kg bw /day) and adolescents of 10 to

17 years of age with 0.54 µg/kg bw/day (95th percentile: 1.07 µg/kg bw /day). The current dietary exposure to acrylamide raises concern for neoplastic effects, across all exposure percentiles and age groups, as the calculated Margins of Exposure (MOE) are substantially below the safety threshold of 10,000. The associated factors and the food contributors to acrylamide dietary exposure were also described by age group [Paper I].

Three methodological approaches to estimate daily BPA exposure were tested using GXXI birth-cohort 13-year follow-up data. The first method followed a traditional deterministic approach, merging the food consumption data with the occurrence data of BPA in food groups. The second and third methods, dietary information (food groups and the packaging material reported) and urinary BPA concentration from a sub-sample, were combined to develop a regression tree model and a random forest, both used to predict in the remaining sample the daily BPA exposure. Random forest was the best methodology to estimate the daily exposure to BPA. The traditional approach was the methodology that was less accurate in estimating BPA daily exposure, with median exposure values in adolescents being 5 to 6 times higher than those measured in urine samples. Despite of the methodological approach, 99.9% of the participants presented an exposure above the Tolerable Daily Intake (TDI) of 0.2 ng/kg bw/day, indicating a potential health risk [Paper II].

In the longitudinal designed study, the median dietary exposure to acrylamide in GXXI birth-cohort ranged from 0.44 µg/kg bw/day at 13 years old (95th percentile: 1.04 µg/kg bw/day) to 0.79 µg/kg bw/day at 4 years old (95th percentile: 1.92 µg/kg bw/day). The MOE for neoplastic effects indicated a public health concern among GXXI participants. A generalised least squares model was used: model 1 adjusted for cross-sectional and longitudinal acrylamide daily dietary exposure; model 2 adjusted for model 1 plus participants' sex, maternal age, maternal education, maternal smoking habits, leisure practice of regular physical activity (cross-sectional and longitudinal); model 3 adjusted for model 2 plus total energy intake (cross-sectional and longitudinal). A positive longitudinal association was found between acrylamide exposure and higher waist circumference (0.06 stdβ; 95%CI: 0.03, 0.08), fat mass percentage (0.04stdβ; 95%CI:0.01,0.06), and blood glucose levels (0.03 stdβ; 95%CI: 0.00, 0.07) (model 3). For blood insulin (0.08

stdβ; 95%CI: 0.05, 0.12) and insulin resistance (0.08 stdβ; 95%CI: 0.05, 0.12), only a significant cross-sectional association was found. In the opposite direction, a negative longitudinal association was found for body mass index z-score (-0.07 stdβ; 95%CI: -0.09, -0.04), total cholesterol (-0.07 stdβ; 95%CI: -0.11, -0.04), LDL-cholesterol (-0.08 stdβ; 95%CI: -0.11, -0.04), HDL-cholesterol (-0.06 stdβ; 95%CI: -0.09, -0.02), z-SBP (-0.07 stdβ; 95%CI: -0.10, -0.03) and z-DBP (-0.03 stdβ; 95%CI: -0.06, -0.00) [Paper III].

At 4 years of age, the median BPA daily exposure was 68.8 ng/kg bw/day (95th percentile: 90.6 ng/kg bw/day), while at 13 years old was 24.7 ng/kg bw/day (95th percentile: 36.3 ng/kg bw/day). According to the exposure values, 99.9% of the participants presented an exposure above the TDI. A generalised least squares model was used: model 1 adjusted for cross-sectional and longitudinal BPA daily exposure predicted values; model 2 adjusted for model 1 plus participant sex, maternal age, maternal education, leisure practice of regular physical activity (cross-sectional and longitudinal); model 3 adjusted for model 2 plus participant height (cross-sectional and longitudinal); and model 4 adjusted for model 2 plus total energy intake (cross-sectional and longitudinal). Blood insulin (0.06 stdβ; 95%CI: 0.03, 0.09) and insulin resistance (0.05 stdβ; 95%CI: 0.02, 0.08) presented a positive longitudinal association with BPA daily exposure after adjustment for model 4. The same findings were observed for fat mass percentage (0.03 stdβ; 95%CI 0.01, 0.06) and waist circumference (0.06 stdβ; 95%CI: 0.04, 0.08). For z-BMI, a positive cross-sectional (0.03 stdβ; 95%CI: 0.01, 0.04) and longitudinal (0.02 stdβ; 95%CI: 0.00, 0.04) association was found [Paper IV].

Finally, the association of food contaminants exposure on pubertal development in children and adolescents aged 4 to 13 was assessed. Linear regression models were used to test the associations between exposure to acrylamide and BPA (individually and combined) and pubertal development. A significant negative association was found between individual acrylamide exposure at 7 years (-0.007β (95% CI: -0.013, -0.002)), 10 years (-0.006β (95% CI: -0.010, -0.003)) and 13 years (-0.005β (95% CI: -0.009, -0.001)) and the pubertal development global score in girls, and a significant positive association for individual acrylamide exposure at 13 years (0.003β (95% CI: 0.000, 0.007)) in

boys. A significant positive association between BPA exposure at 10 and 13 years and a higher pubertal development global score was only found in boys. Testing the combined exposure did not significantly change the results observed for the individual exposure to each food contaminant. Therefore, childhood and adolescent exposure to acrylamide and BPA was associated with impaired timing of puberty onset, representing a relevant public health concern.

Main conclusions

This thesis tested the association between food contaminants exposure, namely acrylamide and bisphenol A, and the development of adverse metabolic outcomes from childhood to adolescence.

Dietary acrylamide exposure was estimated using a probabilistic approach based on self-reported dietary information, while BPA exposure was estimated using three methodologies, with the random forest method (integrating self-reported dietary data and urinary metabolite concentrations) being the most accurate approach. Additionally, we showed that even with a relatively small number of 24-hour urine samples, it is possible to predict a large sample or the population's daily BPA exposure, presenting a relevant insight from this thesis.

The current exposure to acrylamide and bisphenol A in children and adolescents indicated a public health concern since it largely exceeds the values or the margins considered safe.

Longitudinal analyses showed acrylamide exposure positively associated with higher waist circumference, fat mass percentage, blood glucose, insulin, and insulin resistance, while a negative association with higher z-BMI, cholesterol, and blood pressure was found. This variability suggests that the effects of dietary acrylamide exposure on cardiometabolic health are complex and multifactorial.

For BPA, positive associations were found with higher blood insulin, insulin resistance, fat mass percentage, waist circumference, and BMI z-scores from ages 4 to 13. Thus, BPA early exposure may impair cardiometabolic outcomes in future stages of life.

A negative association was found between individual acrylamide exposure and pubertal development in girls at 7 years, 10 years and 13 years. A positive association was found between individual acrylamide and BPA exposure and pubertal development in boys at 10 and 13 years. The combined exposure to acrylamide and BPA did not significantly change the results observed regarding pubertal development. In conclusion, early exposure to acrylamide and BPA, individually or combined, was associated with impaired timing of puberty onset.

Future research should evaluate the effectiveness of mitigation measures and explore consumers' exposure to other food contaminants. From a public health perspective, the example of the European Community may inspire other countries to adopt similar mitigation measures and food policies, protecting consumer health globally. Efforts must focus on reducing food contaminants in products, as well as promoting healthier dietary patterns, mainly targeting children, adolescents, and their caregivers.

RESUMO

Introdução

A alimentação desempenha um papel fundamental na saúde humana, fornecendo energia e nutrientes importantes para um adequado crescimento, desenvolvimento e manutenção da homeostasia do organismo. No entanto, cada vez mais se tem vindo a questionar a ideia simplista de que o aumento da obesidade e de outras comorbilidades é apenas causado pelo desequilíbrio energético. Estudos anteriores avaliaram o efeito da exposição a fatores de risco não convencionais na saúde, como os contaminantes alimentares. A acrilamida e o bisfenol A (BPA) são contaminantes alimentares formados durante o processamento térmico ou que migram dos materiais de embalagem, respetivamente, e que podem afetar negativamente a saúde humana. As crianças e os adolescentes são uma faixa etária vulnerável devido a uma relativamente maior ingestão de alimentos por quilograma de peso corporal e à maturação incompleta dos sistemas metabólicos ou órgãos-chave.

A acrilamida tem a capacidade de formar adutos com proteínas e ácidos nucleicos, provocando genotoxicidade, carcinogenicidade, neurotoxicidade, hepatotoxicidade, toxicidade cardiovascular e toxicidade reprodutiva. Estudos recentes sugeriram que a acrilamida possa ser um potencial disruptor endócrino, alterando biomarcadores cardiometabólicos e o desenvolvimento pubertário, através de mecanismos relacionados com o stress oxidativo, peroxidação lipídica, adipogénese e desregulação hormonal.

O BPA pode interferir com os recetores de estrogénio e, consequentemente, com o sistema endócrino, permitindo que o BPA seja classificado como um disruptor endócrino. Além disso, a exposição crónica a BPA, mesmo em concentrações muito baixas, pode prejudicar a saúde humana, levando ao desenvolvimento de doenças metabólicas e a alterações no desenvolvimento pubertário.

Os estudos epidemiológicos longitudinais que testam a associação entre a exposição a estes contaminantes alimentares e as alterações metabólicas, desde a infância até à adolescência, são limitados e as incertezas ainda necessitam de muitas clarificações.

Objetivos

O principal objetivo da presente tese foi avaliar os efeitos da exposição a contaminantes alimentares, nomeadamente acrilamida e bisfenol A, no desenvolvimento de alterações metabólicas na saúde de crianças e adolescentes. Assim, seguem-se os objetivos específicos a responder:

1. Avaliar a exposição alimentar a compostos xenobióticos derivados do processamento alimentar e de materiais de embalagens alimentar (acrilamida e bisfenol A), aplicando métodos diretos e indiretos:

1.1. Avaliar a exposição alimentar a acrilamida aplicando métodos de avaliação alimentar auto-reportados;

1.2. Avaliar a exposição alimentar a bisfenol A aplicando métodos de avaliação alimentar auto-reportados e metabolitos urinários;

2. Avaliar longitudinalmente a exposição a compostos xenobióticos e testar a associação entre a exposição a contaminantes alimentares e alterações de saúde desde a infância até à adolescência:

2.1. Estimar a avaliação de risco de contaminantes alimentares, nomeadamente acrilamida e bisfenol A;

2.2. Testar longitudinalmente a associação entre a exposição a compostos xenobióticos (acrilamida e bisfenol A) e alterações cardiometabólicas;

2.3. Explorar a associação da exposição a acrilamida e a bisfenol A no desenvolvimento pubertário aos 10 e 13 anos de idade.

Métodos

Esta tese incorpora dados da coorte de nascimento Geração XXI (GXXI) (Porto, Portugal, 2005-2006), bem como do Inquérito Alimentar Nacional e de Atividade Física (IAN-AF 2015-2016).

A coorte de nascimento Geração XXI incluiu na avaliação inicial 8647 recém-nascidos (idade gestacional >24 semanas) de cinco maternidades públicas da área metropolitana do Porto. As avaliações de seguimento foram conduzidas nas idades de 4 (2009–2011, 86% de participação), 7 (2012–2014, 80% de

participação), 10 (2015–2017, 76% de participação) e 13 anos (2018–2020, 54% de participação, interrompida devido à pandemia da COVID-19). Durante cada avaliação, investigadores treinados realizaram entrevistas presenciais usando questionários estruturados, enquanto profissionais de saúde treinados recolheram medidas antropométricas (peso, altura, perímetro da cintura), dados sobre a composição corporal (bioimpedância), pressão arterial, amostras de sangue (colesterol total, colesterol LDL, colesterol HDL, triglicerídeos, glicose, insulina) e amostras de urina (concentração de BPA).

Foi estimada a percentagem de massa gorda (%MG) e o índice de massa corporal específico para a idade e sexo (z-IMC). Calculou-se o modelo de avaliação homeostático para a resistência à insulina (HOMA-IR), para além dos z-scores da pressão arterial sistólica e diastólica específicos para a idade, sexo e altura (z-PAS, z-PAD).

O desenvolvimento pubertário foi avaliado por profissionais treinados aos 10 e 13 anos de idade através da escala de *Tanner*.

Os dados alimentares foram recolhidos através de diários alimentares de três dias (dois dias de semana e um dia de fim de semana), informatizados e categorizados através do sistema de classificação *FoodEx2*. O cuidador principal preencheu o diário alimentar do participante aos 4, 7 e 10 anos de idade, enquanto na avaliação dos 13 anos, o diário alimentar foi preenchido pelo adolescente com a ajuda dos pais/cuidadores.

No inquérito IAN-AF, um total de 5811 participantes com idades compreendidas entre os 3 meses e os 85 anos completaram duas entrevistas. Entre os participantes menores de 18 anos, 1959 forneceram os dados completos relativos a dois dias de registo alimentar. Os nutricionistas treinados recolheram informação adicional sobre diferentes dimensões, nomeadamente características sociodemográficas, dados gerais de saúde, tempo de prática de atividade física de lazer, comportamentos sedentários e antropometria.

A ingestão alimentar foi obtida através de dois diários alimentares de um dia, não consecutivos, para crianças com menos de 10 anos ou de questionários às 24 horas alimentares anteriores para os restantes participantes. Estes dados foram

informatizados através do *Software* eAT24, uma ferramenta validada que integra o sistema de classificação *FoodEx2*.

Resultados

Foi utilizada uma metodologia probabilística para estimar a exposição alimentar diária a acrilamida. Três valores possíveis que representam a distribuição da ocorrência de acrilamida nos alimentos foram atribuídos aleatoriamente a cada item alimentar, tendo em conta a variabilidade intra e inter-individual. As crianças portuguesas dos 1 aos 2 anos apresentaram a mediana mais elevada de exposição alimentar a acrilamida, 0,75 µg/kg peso corporal/dia (percentil 95: 1,41 µg/kg peso/dia), seguidas das crianças dos 3 aos 9 anos com 0,69 µg/kg peso/dia (percentil 95: 1,32 µg/kg peso/dia) e dos adolescentes dos 10 aos 17 anos com 0,54 µg/kg peso/dia (percentil 95: 1,07 µg/kg peso/dia). A exposição alimentar atual a acrilamida levanta preocupações em relação aos efeitos neoplásicos, em todos os percentis de exposição e para todas as faixas etárias, uma vez que as margens de exposição calculadas (MOE) estavam significativamente abaixo do limite de segurança 10000. Os fatores associados e os grupos de alimentos que mais contribuíram para a exposição a acrilamida também foram descritos por faixa etária [Artigo I].

Foram testadas três abordagens metodológicas para estimar a exposição diária a BPA, utilizando dados da avaliação dos 13 anos na coorte de nascimento GXXI. O primeiro método seguiu uma abordagem determinística tradicional, multiplicando o consumo alimentar pela ocorrência (concentração) de BPA nos grupos de alimentos. No segundo e terceiro métodos, a informação alimentar (grupos de alimentos e material de embalagem reportados) e a concentração urinária de BPA medida numa subamostra, foram combinados de forma a desenvolver um modelo de árvore de regressão (*regression tree model*) e uma floresta de árvores aleatória (*random forest*), ambos usados para prever a exposição diária a BPA na restante amostra. O *random forest* foi a melhor metodologia para estimar a exposição diária a BPA. A abordagem tradicional foi a metodologia menos válida e menos precisa para estimar a exposição diária a BPA, sendo os valores medianos de exposição nos adolescentes 5 a 6 vezes

superiores aos medidos na urina. Independentemente da abordagem metodológica, 99,9% dos participantes apresentaram uma exposição acima da Ingestão Diária Tolerável (TDI) de 0,2 ng/kg peso/dia, indicando um potencial risco para a saúde [Artigo II].

No estudo longitudinal, a exposição alimentar mediana a acrilamida na coorte de nascimento GXXI variou de 0,44 µg/kg peso/dia aos 13 anos de idade (percentil 95: 1,04 µg/kg peso/dia) a 0,79 µg /kg peso/dia aos 4 anos de idade (percentil 95: 1,92 µg/kg peso/dia). A MOE para efeitos neoplásicos indicou uma preocupação de saúde pública entre os participantes da GXXI. Foi utilizado o modelo *generalised least squares*: modelo 1 ajustado para a exposição alimentar diária a acrilamida (transversal e longitudinal); modelo 2 ajustado para o modelo 1 mais o sexo dos participantes, idade materna, escolaridade materna, hábitos tabágicos maternos, prática de atividade física regular de lazer (transversal e longitudinal); modelo 3 ajustado para o modelo 2 juntamente com a ingestão energética total (transversal e longitudinal). Encontraram-se associações longitudinais positivas entre a exposição a acrilamida e um maior perímetro da cintura (0,06 stdβ; IC 95%: 0,03; 0,08), %MG (0,04 stdβ; IC 95%: 0,01; 0,06) e glicose no sangue (0,03 stdβ; IC 95%: 0,00; 0,07) (modelo 3). Para a insulina sérica (0,08 stdβ; IC 95%: 0,05; 0,12) e a resistência à insulina (0,08 stdβ; IC 95%: 0,05; 0,12), apenas a associação transversal positiva foi observada. No sentido inverso, foi encontrada uma associação longitudinal negativa para o z-IMC (-0,07 stdβ; IC 95%: -0,09; -0,04), colesterol total (-0,07 stdβ; IC 95%: -0,11; -0,04), colesterol LDL (-0,08 stdβ; IC 95%: -0,11; -0,04), colesterol HDL (-0,06 stdβ; IC 95 %: -0,09; -0,02), z-PAS (-0,07 stdβ; IC 95%: -0,10; -0,03) e z-PAD (-0,03 stdβ; IC 95%: -0,06; -0,00) [Artigo III].

Aos 4 anos de idade, a mediana de exposição diária a BPA foi de 68,8 ng/kg peso/dia (percentil 95: 90,6 ng/kg peso/dia), enquanto aos 13 anos foi de 24,7 ng/kg peso/dia (percentil 95: 36,3 ng/kg peso/dia). De acordo com os valores de exposição, 99,9% dos participantes apresentaram uma exposição acima do TDI. Foi utilizado o modelo *generalised least squares*: modelo 1 ajustado para valores previstos de exposição diária a BPA (transversal e longitudinal); modelo 2 ajustado para o modelo 1 mais sexo do participante, idade materna, escolaridade materna, prática de atividade física regular de lazer (transversal e longitudinal);

modelo 3 ajustado para o modelo 2 juntamente com a altura do participante (transversal e longitudinal); e modelo 4 ajustado para o modelo 2 juntamente com a ingestão energética total (transversal e longitudinal). A insulina sérica (0,06 stdβ; IC 95%: 0,03; 0,09) e a resistência à insulina (0,05 stdβ; IC 95%: 0,02; 0,08) apresentaram uma associação longitudinal positiva com a exposição diária a BPA após ajuste para o modelo 4. Os mesmos resultados foram observados para a %MG (0,03 stdβ; IC 95% 0,01; 0,06) e para o perímetro da cintura (0,06 stdβ; IC 95 %: 0,04; 0,08). Para o z-IMC, foi encontrada uma associação positiva transversal (0,03 stdβ; IC 95%: 0,01; 0,04) e longitudinal (0,02 stdβ; IC 95%: 0,00; 0,04) [Artigo IV].

Por fim, avaliou-se a associação entre a exposição a contaminantes alimentares e o desenvolvimento pubertário em crianças e adolescentes dos 4 aos 13 anos. Foram utilizados modelos de regressão linear para testar as associações entre a exposição a acrilamida e BPA (individualmente e combinados) e o desenvolvimento pubertário. Foi encontrada nas raparigas uma associação negativa entre a exposição individual a acrilamida e o *score* global do desenvolvimento pubertário aos 7 anos (-0,007β (IC 95%: -0,013; -0,002)), 10 anos (-0,006β (IC 95%: -0,010; -0,003)) e os 13 anos (-0,005β (IC 95%: -0,009; -0,001)), e uma associação positiva nos rapazes aos 13 anos (0,003β (IC 95%: 0,000; 0,007)). Uma associação positiva entre a exposição a BPA aos 10 e 13 anos e um *score* global mais elevado no desenvolvimento pubertário foi encontrada apenas nos rapazes. O teste da exposição combinada não alterou significativamente os resultados observados para a exposição individual a cada contaminante alimentar. Assim sendo, a exposição durante a infância e adolescência a acrilamida e a BPA associou-se a um comprometimento do momento do início da puberdade, representando uma relevante preocupação de saúde pública.

Conclusões principais

Esta tese avaliou a associação entre a exposição a contaminantes alimentares, nomeadamente acrilamida e bisfenol A, e o desenvolvimento de alterações metabólicas, desde a infância até à adolescência.

A exposição alimentar a acrilamida foi estimada através de uma abordagem probabilística baseada na informação alimentar auto-reportada, enquanto a exposição a BPA foi estimada através de três metodologias, sendo o método *random forest* (integrando dados alimentares auto-reportados com a concentração urinária de metabolitos) a abordagem mais válida e precisa. Além disso, mostramos que mesmo com um número relativamente pequeno de amostras de urina de 24 horas, é possível prever a exposição diária a BPA para a restante amostra, representado uma importante descoberta desta tese.

A exposição atual a acrilamida e a bisfenol A em crianças e adolescentes indica uma preocupação de saúde pública, uma vez que ultrapassa em muito os valores ou as margens considerados seguros.

As análises longitudinais mostraram que a exposição a acrilamida estava positivamente associada a maior perímetro da cintura, percentagem de massa gorda, glicemia, insulinemia e resistência à insulina, e negativamente associada a um maior z-IMC, nível sanguíneo de colesterol e pressão arterial. Esta variabilidade sugere que os efeitos da exposição alimentar a acrilamida na saúde cardiometabólica são complexos e multifatoriais.

Para o BPA, foram encontradas associações positivas com níveis mais elevados de insulina no sangue, resistência à insulina, percentagem de massa gorda, perímetro da cintura e z-scores de IMC entre os 4 e 13 anos de idade. Assim, a exposição precoce a BPA pode prejudicar parametros cardiometabólicos em fases futuras da vida.

Foi encontrada uma associação negativa entre a exposição individual a acrilamida e o desenvolvimento pubertário em raparigas de 7, 10 e 13 anos. Foi encontrada uma associação positiva entre a exposição individual a acrilamida e BPA e o desenvolvimento pubertário em rapazes de 10 e 13 anos. A exposição combinada a acrilamida e a BPA não alterou significativamente os resultados observados individualmente. Em conclusão, a exposição precoce a acrilamida e a BPA, individualmente ou combinados, esteve associada a uma alteração do momento do início da puberdade.

No futuro, os novos projetos científicos deverão avaliar a eficácia das medidas de mitigação e explorar a exposição dos consumidores a outros contaminantes alimentares. Do ponto de vista da saúde pública, o exemplo da Comunidade Europeia pode inspirar outros países a adoptar medidas de mitigação e políticas alimentares semelhantes, protegendo a saúde dos consumidores a nível global. Os esforços deverão concentrar-se na redução dos contaminantes alimentares nos produtos e na promoção de padrões alimentares mais saudáveis, especialmente dirigidos às crianças, adolescentes e aos seus cuidadores.

INTRODUCTION

1.1. Diet, food quality and food safety

Dites-moi ce que vous mangez et je vais vous dire ce que vous êtes – “Tell me what you eat and I will tell you what you are” – is thought to have been said for the first time by Jean Anthelme Brillat-Savarin in 1826 and remains an unconditional belief to current days. In fact, considering the scientific evidence and the large number of publications on nutritional epidemiology, diet plays an essential role in human health, providing energy and important nutrients to maintain the body's homeostasis (1). On one hand, a safe and high-quality diet may contribute to the prevention of several health disorders. On the other hand, unsafety foods and unhealthy eating habits are risk factors for the development of chronic or non-communicable diseases, namely obesity, diabetes, cardiovascular diseases and cancer (2). However, the simple idea that energy imbalance is the leading cause of the escalating of obesity and other related comorbidities has been increasingly placed into question. Many research teams have recently tested the influence of unconventional risk factors on health, such as environmental chemicals and food contaminants (3). In our diet, contaminants can occur naturally in the food matrix due to environmental contamination, be non-intentionally formed during production, processing, treatment, transport, storage, preparation and cooking, or migrate from packaging materials (Figure 1.1.) (4).

Figure 1.1. *Sources and routes of contamination in the food chain.*

It is essential to highlight that, over many decades, the way humans eat and how products are presented have changed significantly. The use of thermal food processing and packaging materials are examples of achievements that, nowadays, are unimaginable to be reversed. Modern society's trends encourage the search and the use of functional products, increasing the choice of processed or packaged foods that allow one to prepare meals in minutes (5).

Thermal food processing not only allows food safety by eliminating possible pathogenic agents but also improves the nutritional properties of foodstuffs, increases the bioavailability of many compounds and creates desirable sensory characteristics, such as flavours, aromas, colours and texture (6,7). Despite this, with thermal processing extension, non-intentional heat-induced hazardous substances are also formed (7,8). In the last few years, several publications estimated the exposure and tested the safety of these food contaminants in health-related outcomes (9–11). According to these findings, international organisations have published scientific opinions, highlighting a public health concern due to their probable adverse impact on the organic system (12–15).

Regarding food packaging, the primary purpose of this technology is to ensure the safety of food products, protecting them against physical, chemical and biological hazards. Thus, it is possible to increase the shelf-life, retard food deterioration, maintain the food quality and food safety and, consequently, reduce the food waste (16). Food packaging provides product labelling, including nutritional information and cooking instructions, facilitates tracking throughout the entire food chain, supports relevant information, and appeals to consumers to purchase (17). Considering all these reasons, packaging plays an essential role in maintaining foodstuff characteristics in transportation and storage until the final consumer. In Portugal, findings from the National Food, Nutrition and Physical Activity Survey 2015-2016 (IAN-AF 2015-2016) showed that 57% of the consumed food items were packaged, being plastic the most frequent packaging material reported (69%), followed by multilayer material (14%), glass (9%), paperboard/paper (4%), metal (3%), and other materials (1%) (18).

However, to ensure the technological properties of the package, several additives and hazardous substances are usually added to the raw packaging materials or are present in the adhesives, coatings, inks, dyes and lids (4,19,20). The interaction between the

physical and chemical properties of packaging materials and the food components may lead to the transfer of certain substances into food and beverages (21,22). This process, known as migration, contributes to exposure to food chemicals that may adversely affect human health and requires monitoring and control (19).

1.2. Risk assessment of food hazards

Access to quality and safe food is a right imprinted in the Universal Declaration of Human Rights (23) and in the General Food Law Regulation (24). Thus, in 1968, the Food and Agriculture Organization of the United Nations and World Health Organization published the first version of Codex Alimentarius, compelling a collection of food standards and related texts to protect consumers' health and ensure air practices of international food commercialisation (25). This document has been updated since that date, always aiming to contribute to the safety, quality and fairness of the food system, including new food standards, guidelines and codes of practice regarding different issues, such as food hygiene, food additives, residues of pesticides, veterinary drugs and contaminants (26). Despite their application being voluntary, as they are recommendations, Codex standards attended as a basis for national legislation of several countries.

According to Codex Alimentarius, within the risk analysis of food hazards, risk assessment is essential to apply scientific knowledge in establishing food safety standards, guidelines and recommendations that will support decision-making. A food hazard is any biological, chemical or physical agent in the diet that can potentially cause an adverse health effect in the individual organism'. To evaluate the risk associated with exposure to certain food hazards, such as food contaminants, the risk assessment approach uses available scientific data. It follows four steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation (26). By definition, hazard identification is *the identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods;* hazard characterisation is *the qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical, and physical agents which may be present in food*; exposure assessment is *the qualitative and/or quantitative evaluation of the likely intake of*

biological, chemical, and physical agents via food as well as exposures from other sources if relevant; and risk characterisation is *the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterisation and exposure assessment* (26).

1.2.1. Hazard identification and hazard characterisation

In risk assessment studies, hazard assessment steps aim to evaluate the adverse effects relevant to human health resulting from substance exposure, and characterise the dose-response relationships for the selected outcomes (27).

In hazard identification, researchers identify the contaminant present in food, the potential critical endpoints and justify the relevance of their study (27). As most previous studies were performed in animals, adverse endpoints should be critically selected considering statistical procedures, biological plausibility, and biological relevance (27).

In the hazard characterisation step, the health effects resulting from exposure to food hazards are assessed, the dose-response is deeply explored, and a safe exposure level for consumers is calculated. In other words, the final purpose of hazard characterisation is to establish a value of intake (reference dose) for which an adverse health effect on the consumer is confidently not expected (27). For this, uncertainty, variability inter- and intraspecies, suboptimal study characteristics and missing data are critical factors that must be considered (27). In a more realistic scenario, statistical analysis uses a range of dose levels, providing data regarding the lower and higher sections of the dose-response relationship (27). Two different statistical approaches can be applied to determine the reference dose: No-Observed-Adverse-Effect Level (NOAEL) and Benchmark Dose (BMD) approach (27).

The NOAEL, gathered from previous publications, is the highest dose tested in a specific study for which there was no evidence of an adverse effect for a particular outcome. Consequently, NOAEL is largely affected by the selected dose ranges, sample size, study power, and research expert judgement and their decisions (27).

The BMD approach uses all potential toxicological effects and all dose-response data to model the dose-response curve for a specific endpoint. The BMD is the dose responsible for a defined change in the compound's response, using the control group as a reference. The reference dose in this approach is the lower value of the confidence limit of the BMD (BMDL), usually considered at 95% (Figure 2.1.) (27).

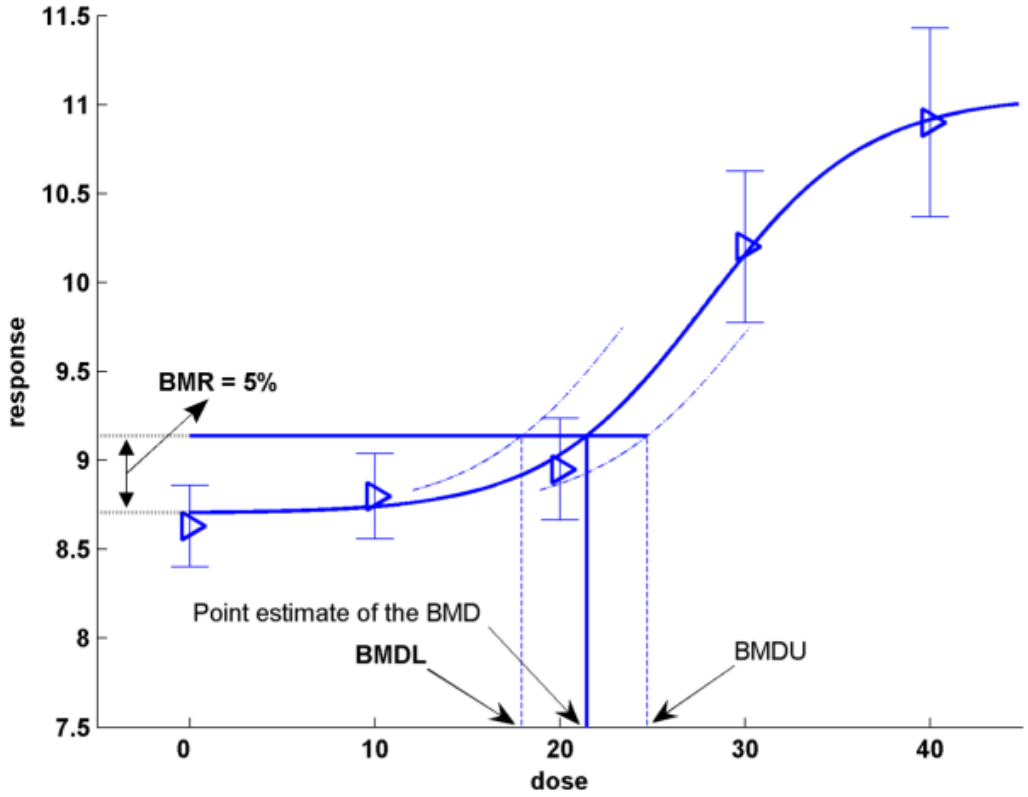

Figure 2.1. *Key concepts for the BMD approach are illustrated using hypothetical continuous data and for a change in response of 5%. Source: EFSA, 2009 (28).*

The advantage of the BMD approach over the NOAEL approach relies on the account of all confidence interval ranges for all relevant endpoints, considering uncertainties in the data. In contrast, the NOAEL approach often does not adequately cover all experimental uncertainties, such as the low power of the study, which can lead to a reference dose that may be significantly overestimated (27).

Reference doses will then be used for establishing health-based guidance values, namely acceptable daily intakes (ADIs) for food additives and pesticide residues and tolerable daily intakes (TDIs) or tolerable weekly intakes (TWIs) for food contaminants (27).

Nevertheless, if data for establishing health-based guidance values are considered inadequate but allow the estimation of a reference dose ($BMDL_x$), a margin of exposure (MOE) approach must be applied in the following steps. Besides data gaps, this approach is also used for genotoxic and carcinogenic chemicals, for which it is generally believed that any level of exposure is harmful (29).

1.2.2. Exposure assessment and risk characterisation

After hazard identification and hazard characterisation, exposure assessment and risk characterisation are crucial steps for publishing rigorous, relevant and transparent scientific opinions on public health concerns related to specific food contaminants.

The scope of the exposure assessment is to assess chronic exposure to food contaminants. The importance of selecting the appropriate method to estimate the exposure assessment is undoubted, as it depends on the study's purpose, the nature of the contaminant, the available data, and the available resources (30). As an indirect method, theoretical daily dietary exposure is the approach most commonly used and combines food consumption data with estimates of chemical concentration in foodstuffs. This general model can be represented as:

$$Dietary\ exposure\ (\mu g/kg_{body\ weight}/day) = Food\ consumption\ (kg_{food}/kg_{body\ weight}/day) \times Contaminant\ concentration\ (\mu g/kg_{food})$$

Individual food consumption data can be collected through self-reported measures, namely, 24-hour recalls and food diaries, while food contaminant concentration in food products can be obtained by laboratory analysis or gathered from the literature previously published (22,30). However, self-reported food consumption information has several limitations related to misreporting, recall bias, participation bias, and identification of all dietary sources of food contaminants (31–33). For example, the prevalence of misreporting in the Portuguese adult population was 29.9%, with under-reporting accounting for the majority of cases (28.5%) (34). Furthermore, depending on the hazard compound, dietary assessment methods may be insufficient to account for intra-individual variability in usual food intake (35) and, consequently, fail to capture the entire picture of food contaminants exposure. Thus, to overcome some of these limitations, different types of models may be applied to combine consumption and

concentration data: deterministic models, using a single estimate value for each variable; simple distributions, counting only with the variability of food intake and using a fixed value for the contaminant concentration variable; and probabilistic models, considering the variability (i.e. heterogeneity) and the uncertainty (i.e. missing information) of the variables' distribution (30).

Using direct methods, human food contaminants exposure can be evaluated by measuring the concentration of the hazard compound, or its metabolites, in biological samples. Biomarkers, such as urinary metabolites or haemoglobin adducts, provide objective estimates and may also be used to validate and improve indirect methods, especially the probabilistic models. Moreover, biomarkers account for all sources of exposure, not only with daily dietary exposure. However, in addition to the higher associated costs of direct methods, they are challenging to implement and have specific limitations (36). For example, depending on the nature of the hazard compound, they may represent chronic or acute intakes, leading to over or underestimating real individual exposure (30) whenever a biological sample is not properly selected.

Thus, researchers must consider a broad set of factors and intake determinants to make informed decisions based on scientific evidence, ensuring an accurate integration of food consumption information with data on the contaminant's occurrence while evaluating a scenario representative of the real-life situation.

As a final step, risk characterisation integrates the evidence from hazard identification, hazard characterisation and exposure assessment to obtain an estimated risk in a given population. Here, the exposure level is compared with the health-based guidance values derived from hazard characterisation (ADIs, TDIs, TWIs), indicating the proportion of the population at risk of potential adverse health effects (36,37). Considering the available data and the food contaminant, when the MOE approach is the best option, the ratio between the reference value ($BMDL_x$) and the individual's exposure dose will indicate the public health concern.

$$MOE = \frac{BMDLx\ (\mu g/kg_{body\ weight}/day)}{Dietary\ exposure\ \left(\mu g/kg_{body\ weight}/day\right)}$$

Nevertheless, a meaningful risk characterisation of a food contaminant can only be achieved by considering all the uncertainties associated with hazard and exposure

assessments (37). Consequently, public health researchers can draw conclusions on the risk level for the general population or specific groups and help political decision-makers to advise consumers or to control food production.

1.3. State of the art on food contaminants

As mentioned previously in Chapter 1.1, food and beverages are important route sources of contaminants that may occur during production, storage and processing or migrate from packaging materials. Thus, during the life course, humans are usually exposed to a large variety of exogenous contaminants through dietary intake, acutely or chronically, impairing health outcomes in future stages of life. Moreover, in light of current knowledge, some limited evidence shows that food contaminants may interact or compete between them and/or with other components of the food matrix, either exacerbating or reducing the overall health effects of each substance (38).

The extraordinary chemical complexity of foods largely limits the complete identification of all components and food contaminants in nutritional epidemiology. In fact, it is estimated that 99% of the biochemicals in our diet, playing a role in the relation health-disease, remain unknown and untracked by the national food safety organisations and public health institutes (39).

The present thesis will focus on acrylamide and bisphenol A due to their importance for food contaminants resulting from food processing and package materials, respectively. Considering their origin and occurrence level, humans are inevitably subjected to chronic exposure to these food contaminants, even at low doses. Furthermore, the growing public health concerns regarding acrylamide and bisphenol A exposure have underscored the urgent need for updated risk assessment studies, mainly focusing on vulnerable population groups, to safeguard future generations from the potentially harmful effects of these compounds.

1.3.1. Acrylamide

Industrial and home-cooking thermal procedures, namely roasting, toasting, baking and frying, are cooking methods widely applied to our kitchens to ensure safety,

nutritional proprieties and desirable sensory characteristics of foodstuffs, such as flavours, aromas, colours and texture (6). Despite the theoretically strong link of the Portuguese diet to a Mediterranean food pattern, with soups, boils and stews being the most characteristic cooking method (40,41), the prevalence of roasting, toasting, baking and frying was highly relevant according to the data collected in IAN-AF 2015-2016 (42).

However, the thermal process is also responsible for the non-intentional production of hazardous substances. Acrylamide is one of these food contaminants formed during the high thermal processing of specific foodstuffs. This contaminant was found in food products for the first time in 2002 by the Swedish Authorities (43). As other heat-induced hazardous substances, acrylamide is formed at temperatures higher than 120ºC, in low moisture conditions, through the chemical reaction between amino acid asparagine and reducing sugars, such as fructose and glucose, by the extension of the Maillard reaction (8,12). Consequently, food products rich in carbohydrates, such as cereal products (bread, toasts, breakfast cereals, biscuits and snacks), potato products (roasted potatoes, fried potatoes, potato chips, French fries and crisps), coffee and coffee substitutes, usually present higher concentrations of acrylamide (14).

Humans are exposed to acrylamide through diet, dermal absorption, or inhalation related to occupational settings and tobacco smoke. However, diet is the most important source of acrylamide for the general non-smoker population (14,44). Furthermore, exposure is strongly influenced by eating patterns and cooking practices of different geographical regions, thereby impacting the development of adverse health effects (3,45,46). Interest in the health impacts of acrylamide exposure has increased significantly in the last two decades, driven by its identification as a public health concern by different international organisations (14,47). Consequently, and because acrylamide was found to have significantly higher concentrations in some foodstuffs compared to analogue products of the same category, in 2017, the European Commission (EC) introduced Regulation No 2017/2158, establishing mitigation measures and benchmark levels to reduce the occurrence of acrylamide in foods (48).

This document, mandatory for all European Member States and directly applicable to food business operators, defined benchmark levels of acrylamide allowed for specific foods, including French fries, potato-based products, bread, breakfast cereals, fine

bakery wares, coffee, coffee substitutes, and baby food, emphasising that occurrence should be as low as reasonably possible (48). Additionally, the regulation proposed different mitigation measures, covering all phases of food production susceptible to acrylamide formation, with guidelines for agronomy practices, raw material selection, storage and transportation, recipe development, process design, and available information to the consumers (48). These measures were adapted to the nature of the food operation and grounded in scientific and technical knowledge. They have proven effective in reducing acrylamide levels without compromising the quality and safety of the food product (48) and are aligned with other publications (49,50).

Despite these mitigation measures, human exposure to acrylamide increased until 2017, and it has been hypothesised that exposure levels may still be very high in our days (46). From a public health perspective, the precautionary principle should be followed, combining efforts to reduce acrylamide exposure through the diet. This includes encouraging the food industry to implement regulations and guidelines that keep acrylamide levels as low as possible and also promoting consumer shifts for better dietary choices.

1.3.2. Bisphenol A

Currently, food safety from farm to fork is ensured by the EC policies, making comprehensive information available to every citizen regarding the production, processing, packaging, labelling, and sale of the food they consume. Thus, the high level of protection of human, animal, and plant health is the main goal of the EC Food Safety policies (51).

Across all sectors of the food chain, food and drinks are in contact with a large number of materials before reaching the final consumer. Such materials include containers, vats, machinery, kitchenware, tableware, and food packaging, called Food Contact Materials (FCM). Considering the transfer of FCM components into foodstuffs and their potential negative impact on food quality and human health, the EC published two Regulations to guide manufacturing practices of FCM production and guarantee their chemical safety (52,53).

As a monomer, bisphenol A (BPA) was authorised to be used in the production of polycarbonate plastics and epoxy resins of FCM (54), as well as in other non-food-related applications, namely toys, pacifiers, medical devices, thermal paper, printing inks, electronic products, and flame retardants (55–57). Thus, nowadays, BPA is frequently found in reusable beverage bottles, infant feeding bottles, tableware, cookware, microwave ovenware, food containers, and reservoirs for water dispensers made with polycarbonate plastics (56,57). Moreover, cans, vats, tanks and supply systems in contact with foods and drinks are coated with protective linings containing epoxy resins composed of BPA (56).

BPA can leach the components of FCM and migrate into foods and beverages in a process called migration. This process is influenced by storage conditions such as temperature, pH of surrounding medium, water content, area and time of contact (58,59). Therefore, the most important source of BPA exposure for the general population is diet (56,60), primarily through canned food and beverages (61,62).

Given the raised concerns about the adverse health effects of BPA exposure, even at low doses, the EC regulated the use of BPA in FCM (54) and banned the use of BPA in all infant feeding bottles (63) based on the precautionary principle. Moreover, in 2018, the EC updated the specific migration limit of BPA from 0.6 mg/kg to 0.05 mg/kg of food (64). Despite the decrease observed in the last years, BPA exposure remains very high worldwide (65,66). In light of the current knowledge and taking into account the uncertainties, the public health concern cannot be disclosed for all population groups (56). Thus, very recently, in December 2024, the EC approved a new Regulation 2024/3190, amending Regulation No 10/2011 and repealing Regulation 2018/213, prohibiting the use of BPA in the manufacture of FCM and articles of which it may be a component, including adhesives, rubbers, ion-exchange resins, plastics, printing inks, silicones, varnishes and coatings (67). Moreover, the document stated that other bisphenols and bisphenol derivatives, such as bisphenol S, are not permitted without an up-to-date assessment by the European Food Safety Authority (EFSA), showing that their use does not endanger human health. In case of a specific application where suitable alternatives do not exist and in which such substances are critical for the purpose of that FCM, business operators should apply for authorisation to use hazardous bisphenols or bisphenol derivatives. This Regulation will enter into force on 20th January 2025. Since the prohibition of BPA use presents a significant

challenge in the specific formulation of FCM and articles, EC allowed a transition period of 18 months on which business operators have to find alternative solutions that should not represent a health risk to consumers (67).

1.4. Diet and food contaminants exposure through the life course

Regardless of each country's unique dietary and cultural identity, significant changes in eating patterns and lifestyle behaviours have been observed throughout the globe during the last decades (68). The continuous development of technology related to food production and processing, regional urbanisation, economic globalisation, the mass media revolution, and income improvement are the most critical factors that largely contributed to shifts in the global food system and food consumption (69). Even though we know that achievements in food technology were essential to improve food availability, digestibility, safety, transportability, storage life, and food waste, this accelerated process may present implications for human nutrition and health (70). Nowadays, the durability, convenience of ready-to-eat meals, accessibility, palatability, marketing, and price make highly processed products very tempting over fresh food at the moment of purchase decision in both developed and developing countries (69,71). Furthermore, these shifts in dietary patterns and lifestyle behaviours have been associated with the increasing prevalence of non-communicable diseases, such as obesity, type-2 diabetes, cardiovascular diseases, and cancer, representing in our days a major global socioeconomic burden (69).

It is also important to consider that each person can theoretically change or improve their eating habits in any life stage, modifying their exposure to food contaminants. However, in specific time periods, individuals are more prone to experience significant dietary changes, called inflexion points (72). Early life, i.e., the first 1000 days of life, childhood, and adolescence, are critical temporal windows when dietary patterns are likely to change due to social and cultural influence as well as socioeconomic and environmental factors (72,73). In fact, early food preferences, nutritional quality, and food safety are crucial for establishing long-term health behaviour patterns, thereby modulating the risk of developing non-communicable diseases (72,74).

A higher exposure to food contaminants may result from specific dietary patterns observed in children and adolescents (75,76). In addition, children consume more

foodstuffs expressed per kg of body weight than adults, relatively increasing the adverse compound exposure (77). Beyond that, as they are still growing, the physiology and some organ systems of children and adolescents present an incomplete maturation, making these population groups more susceptible to neurological, endocrine, and immunological toxic effects (75,78).

For these reasons, children and adolescents are recognised as a vulnerable group to the detrimental health effects of food contaminants. Even though most of the studies that assessed the risk of food hazards were focused on and performed on adults. To fill this knowledge gap and empower the discussion between public health researchers, risk communicators, and policymakers, more epidemiological studies are needed to clarify the uncertainties regarding the effects of early food contaminant exposure on future health outcomes.

1.5. Health effects of food contaminants exposure

To fully understand the mechanisms involved in the effects of food contaminants exposure, the toxicokinetics of the compound should be comprehensively explored. Toxicokinetics describes all processes of absorption, distribution, metabolism and excretion and is essential to understanding the mode of action of food contaminants and their metabolites (79).

1.5.1. Acrylamide

After oral exposure, acrylamide is completely absorbed in the gastrointestinal tract. Reaching the systemic circulation, acrylamide is distributed to different tissues throughout the body. Independently of the route source, acrylamide is extensively metabolised through its conjugation with glutathione or its epoxidation to glycidamide in the cytochrome P450 enzyme complex. Then, acrylamide and glycidamide can form covalent binding with proteins, including haemoglobin, making these important blood biomarkers of all acrylamide exposure sources. The metabolic inactivation of acrylamide and glycidamide comprises the hydrolysis of glycidamide or the formation of glutathione adducts for both compounds, which are then transformed into

mercapturic acids and excreted in urine (80,81). The concentration of these mercapturic acids in urine can be used as biomarkers of all acrylamide exposure. The body elimination appears to occur in two phases, being the half-live for the first phase of 5 to 8 hours and the second of 8 days (80). Finally, bioaccumulation is not the most probable scenario despite their covalent binding to specific proteins.

Regarding the modes of action, acrylamide can react with proteins due to their electrophilic characteristics. Effectively, acrylamide and glycidamide can form adducts by binding to nucleophilic sites in proteins (haemoglobin, albumin, enzymes) and nucleic acids (DNA), impairing the correct function of different cells, such as neuronal cells, immune cells, and germ cells inducing, ultimately, apoptosis (82–85). Therefore, this food contaminant may cause different toxicological effects, namely genotoxicity, carcinogenicity, neurotoxicity, hepatoxicity, cardiovascular toxicity and reproductive toxicity (14,49,50,86).

Considering the available toxicological studies, the International Agency for Research on Cancer classified acrylamide as probably carcinogenic in 1994 (47). In 2015, EFSA published a Scientific Opinion on acrylamide exposure from foods, which reviewed a large number of epidemiological data from animal and human studies, identified the critical effects and derived a health-based guidance value (14). To the best of their knowledge and considering all data published until 2015, EFSA identified four possible critical endpoints: neurotoxicity, effects on male reproduction, developmental toxicity and carcinogenicity. As both acrylamide and glycidamide were positive for genotoxicity tests, the EFSA Panel defined a Lower confidence limit of the Benchmark Dose for a 10% extra risk ($BMDL_{10}$), based on animal studies, to be used for the risk characterisation, applying the MOE approach.

Thus, for the non-neoplastic endpoints (neurotoxicity, reproductive toxicity, and developmental toxicity), EFSA followed a conservative approach where the most relevant and sensitive outcome was considered. This approach identified peripheral neuropathy as the critical effect, corresponding to the lowest derivated $BMDL_{10}$ value of 0.43 mg of acrylamide/kg of body weight/day. A MOE lower than 125 indicated a public health concern for this outcome. For neoplastic effects, a $BMDL_{10}$ value of 0.17 mg of acrylamide/kg of body weight/day was derived from the incidence of Harderian gland adenomas and adenocarcinomas (organ found in mice), corresponding to the

lowest value of the available genotoxic and carcinogenic data. A MOE lower than 10,000 indicated public health concern for genotoxic and carcinogenic effects (14).

At the end of the Scientific Opinion, EFSA concluded that the exposure to acrylamide across European countries and age groups was above the MOE of 125, indicating no health concern for non-neoplastic effects. However, in toddlers and older children, the 95th percentile of exposure for the most conservative scenario presented a MOE of 126 and 134, respectively, very close to the limit considered safe. Regarding neoplastic effects, since the MOE values were substantially lower than 10,000, the EFSA Panel concluded that there was a public health concern across all countries and age groups (14).

Recently, the potential endocrine disruptor effect of acrylamide has been discussed, as well as its role in oxidative stress (3,87,88). On one hand, different authors concluded that acrylamide may impair the balance of cardiometabolic biomarkers, such as serum lipid and glucose profiles, through a not completely understood mechanism involving lipid peroxidation and adipogenesis (3,9,89,90). On the other hand, studies performed on adults found controversial results by testing the association of acrylamide exposure with obesity and obesity-related comorbidities (91–93). For example, in National Health and Nutrition Examination Surveys (NHANES) with national cross-sectional data from adults in the United States, positive, negative or non-significant associations were found between acrylamide exposure and cardiometabolic outcomes, namely lipid profile and body composition outcomes, according to the blood biomarkers chosen to characterise exposure (94–98). Also, the results from a cross-sectional study performed on adults from the European Prospective Investigation into Cancer and Nutrition cohort (EPIC) showed a positive association of acrylamide exposure with Body Mass Index (BMI), considering haemoglobin adducts of glycidamide and the ratio glycidamide-to-acrylamide but failed to find an association for haemoglobin adducts of acrylamide (93). Lastly, concerning glucose homeostasis, a study of Chinese adults found a positive association between acrylamide exposure and higher fasting plasma glucose levels (90). However, an NHANES study found inconsistent results for type 2 diabetes, depending on the chosen exposure biomarker (96).

Acrylamide may also cause detrimental effects on the reproductive system, impairing pubertal development. But, the mechanisms behind this adverse association are not entirely understood, and different plausible pathways exist. On the one hand, animal experiments demonstrated that oral exposure to acrylamide increased reactive oxygen species and DNA damage, leading to meiotic division abnormalities, cell inviability and apoptosis of germ cells (99,100). On the other hand, acrylamide also interacts directly with the reproductive neuroendocrine system by disrupting sex hormones. Acting as an endocrine-disruptor chemical, acrylamide disturbs the hypothalamic-pituitary-gonadal axis by interfering with the hypothalamus-produced gonadotropin-releasing hormone neurons and downregulate sex hormone concentrations in both female and male pubertal animals (101). Additionally, epidemiological evidence assessing the association between dietary acrylamide exposure and pubertal development is very limited and inconclusive, especially in the human adolescent population. Among a preschool-age Japanese sample in 2006, a positive association between dietary acrylamide exposure and sex hormone levels was found only in boys (102). Another Japanese study developed during 2013-2015, including adolescents aged 13 to 14, found that acrylamide mercapturic acids were associated with lower testosterone levels and lower puberty stage in males (103). From two studies performed with different cross-sectional data from NHANES, one with data from 2003-2004 failed to find significant results (104), but the other with data from 2013-2016 found a negative association of haemoglobin adducts of acrylamide and glycidamide with sex hormone levels in both girls and boys (101).

1.5.2. Bisphenol A

BPA is rapidly absorbed in the gastrointestinal tract after oral exposure, with a high degree of absorption (105). In the liver, BPA is metabolised primarily through glucuronide conjugation or by sulfate conjugation to a lower extent, inactivating this contaminant (105). Then, the biologically inactive form of BPA reaches the systemic circulation, where is rapidly distributed in the entire body with no particular affinity for any organ. Conjugated BPA is excreted primarily via urine (105), with a half-life of nearly 6 hours in the human body, being this a valid biomarker of daily exposure (106,107). Considering the lower contribution of other routes, the concentration of BPA

in urine may represent dietary exposure to this contaminant for the general population (57,108,109). Finally, toxicokinetic data show that despite the hydrophobic characteristic of BPA, there is no evidence of its accumulation in the environment or biological tissues (105,110).

Glucuronidated BPA may interfere with oestrogen receptors and, consequently, with the endocrine system, allowing BPA to be classified as an endocrine disruptor (105,111). Moreover, different studies showed that chronic BPA exposure may impair human health, even at lower concentrations, leading to a broad set of non-communicable diseases (112,113).

In 2015, considering the publications available on the health effects of BPA dietary exposure, EFSA established a temporary Tolerable Daily Intake (t-TDI) of 4 µg/kg of body weight/day, pending the results of long-term studies, which was expected to be published in a short period (114). Later on, in 2023, EFSA re-evaluated the risk related to the presence of BPA in foodstuffs and included the most recent publications and the results from the pending longitudinal studies. In this scientific opinion, according to the available epidemiological data performed in animal models and humans, EFSA identified different potential endpoints of BPA exposure: general toxicity, including, among others, body weight, liver, kidney and thyroid gland effects, metabolic disorders, neurotoxicity, cardiotoxicity, carcinogenicity, immunotoxicity, reproductive and developmental toxicity (56). Only endpoints with a likelihood of “Likely” or “Very Likely” classified in the weight of evidence analysis were included to derivate TDI. As a result, only studies performed on animals were included related to the immunotoxicity, metabolic effects (uric acid), neurotoxicity, and reproductive and developmental toxicity endpoints (56). Thus, considering the uncertainties of the included studies, EFSA updated the TDI value to 0.2 ng/kg of body weight/day, based on a study of Th17 cells immunotoxicity, as the most relevant health effect of oral BPA exposure (56,115). Finally, EFSA concluded that dietary BPA exposure across all age groups represented a public health concern since it vastly exceeded the updated TDI value (56).

Regardless of the findings of experimental animal studies, the results of human epidemiological studies remain inconclusive, especially regarding anthropometric and cardiometabolic outcomes. In different cross-sectional studies, including data from children and adults, cardiometabolic outcomes such as increased BMI (116–118),

waist circumference (116), blood glucose (116,119) and serum lipids (120) were positively associated with BPA exposure. However, other cross-sectional studies found contradictory results or no association between BPA exposure and the same outcomes, also performed in both children and adult population groups (65,121–123). A systematic review with meta-analyses published in 2020 found a significant positive association between exposure to BPA and overweight and general and abdominal obesity in adults. Still, it failed to find an association across children (124). Recently, an umbrella review concluded that BPA exposure was associated with multiple health-related outcomes, including obesity and cardiovascular risk factors. However, interpreting these results should be done cautiously given the high heterogeneity and the low effect size observed in the included systematic reviews (125). In fact, the majority of the previous meta-analyses included studies with both cross-sectional and cohort designs, most of the time without stratifying the results by age group.

As an endocrine disruptor compound, BPA interferes with the normal function of the reproductive system, negatively affecting pubertal regulation at younger ages. Considering the estrogenic and anti-androgenic potential effects of BPA, this food contaminant affects the pubertal timing by disturbing the secretion of sex hormones (111,126). However, previous studies evaluating the effects of BPA exposure on pubertal development have reported conflicting results, leaving the nature of this association unclear. From a cohort sub-sample of girls, prenatal exposure to BPA was inversely associated with early breast budding, but the authors failed to find that association with childhood exposure (127). A cross-sectional study performed on Chinese girls aged between 6 and 9 years found that higher levels of urinary BPA were positively associated with precocious puberty (128). However, some studies have found no significant association, as concluded in different systemic reviews (126,129,130). Indeed, two different meta-analyses, published in 2020 and 2022, concluded that BPA exposure was not associated with changes in pubertal development despite the large heterogeneity among studies (sample size, study designs, exposure assessment methodology and statistical analysis) (126,130).

1.6. Food contaminants and uncertainties

In every toxicological study, and for the purpose of this thesis, some uncertainties be addressed. First, the exposure assessments were frequently estimated using occurrence data of contaminants in food or dietary intake information that may not represent the current exposure or the current consumption, considering the large time gap between the data collection and the performed study. Second, most studies assessing the exposure to food contaminants applied deterministic approaches, not considering the intra or interindividual variability. Third, the impact of other dietary components on the toxic effects of food contaminants was not commonly considered (39). Fourth, indirect methods are used to estimate food contaminants exposure, and direct methods are used to test the associations with health outcomes, not integrating both methodological approaches. Fifth, the data used by EFSA to perform the dose-response assessment and to derivate safe values only included studies on animals since the human studies were not available or were not adequate to establish reference points (14,56). Sixth, EFSA uses the Harderian gland as the target tissue for the hazard characterisation of acrylamide when this organ is not found in the human body (14). Seventh, the majority of the studies testing the associations were made in animal models or with adults, with the research on children and adolescents being very limited. Eighth, the inconsistent results found across different epidemiological studies testing the association between food contaminants exposure and health outcomes. Ninth, the lack of studies assessing the association of acrylamide exposure with non-carcinogenic outcomes, such as endocrine, cardiometabolic and obesity-related endpoints. Tenth, the multiple causes behind the development of non-communicable diseases challenge the founds observed by isolating a single food contaminant effect. Eleventh, the time frame of the previous studies may not be sufficient to identify or clearly find associations between food contaminant exposure and health effects, especially in children and adolescents. Twelfth, most previous studies were cross-sectional and not representative of the population where they were conducted. Thirteenth, longitudinal studies published assessing the effect of food contaminants on health outcomes were very rare, especially considering the exposure in the early stages of life, namely during childhood and adolescence.

1.7. Conceptual framework and justification

The complexity of the diet and the difficulty in establishing a clear diet-disease relationship is unquestionable. Early exposures to hazardous compounds have a negative impact on health outcomes in future stages of life, with effects that may only become detectable during adolescence.

Considering the previous publications and the uncertainties described in Chapter 1.6. there is a lack of risk assessment studies that consider both direct and indirect methods to estimate dietary exposure to food contaminants and apply probabilistic approaches. Moreover, to address the knowledge gap regarding exposure to a mixture of hazardous compounds, more studies are needed to assess the effect of multiple food contaminants intake on human health. Finally, longitudinal studies, especially those performed in younger population groups, are required to clarify the associations between food contaminants exposure and health effects and their causality.

In this line, the present thesis will focus on exposure assessment by applying different methodological approaches and considering consumption information and data from biomonitoring. To clarify the relationship between food contaminants and health outcomes, this work will be longitudinal and designed from childhood to adolescence, combining exposure to different food contaminants.

With this in mind, the present results will be essential for the definition of future scientific publications and future research pathways, considering the health concerns identified. Furthermore, our findings will contribute to identifying and developing new indicators that can support public health professionals, risk communicators, and policymakers in formulating effective health policies.

OBJECTIVES

The main objective of the present thesis was to assess the effects of food contaminants exposure, particularly acrylamide and bisphenol A, on the development of metabolic outcomes in children and adolescents' health. Thus, the specific objectives to be answered were:

1. To assess dietary exposure to xenobiotic compounds derived from food processing and packaging materials (acrylamide and bisphenol A), applying direct and indirect methods:

1.1. To assess dietary exposure to acrylamide by applying self-reported dietary assessment methods (paper I);

1.2. To assess dietary exposure to bisphenol A by applying self-reported dietary assessment methods and urinary metabolites (paper II);

2. To longitudinal assess the xenobiotic compounds exposure and to test the association of food contaminants exposure with health outcomes from childhood to adolescence:

2.1. To estimate the risk assessment of food contaminants, namely acrylamide and bisphenol A (papers III and IV);

2.2. To longitudinally assess the association between xenobiotic compounds exposure (acrylamide and bisphenol A) and cardiometabolic outcomes (papers III and IV);

2.3. To assess the association of acrylamide and bisphenol A exposure on pubertal development at 10 and 13 years old (paper V).

RESEARCH METHODS

For this thesis, data from the Generation XXI (GXXI) population-based birth cohort, established in the metropolitan area of Porto between 2005 and 2006 (131), and data from the National Food, Nutrition, and Physical Activity Survey of the Portuguese general population conducted in 2015-2016 (IAN-AF 2015-2016) (132) was used. Considering these two datasets, prospective data from GXXI collected at the ages of 4, 7, 10 and 13, as well as cross-sectional data from the IAN-AF 2015-2016 of the Portuguese population aged between 3 months and 84 years, were used for the design and development of the manuscripts included.

The following sections provide a general overview of the study design, participants characteristics and data collection methods for each study. The selection of eligible participants was determined by the specific research objectives and is detailed in the methods sections of the individual papers.

Ethics approval and consent to participate

All phases of both studies complied with the national legislation with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki (133).

For the GXXI birth cohort, the baseline and follow-up evaluations were approved by the University of Porto Medical School/ S. João Hospital Centre Ethics Committee, except the 13-year follow-up that was approved by the ISPUP Ethics Committee. At baseline and follow-up evaluations, all procedures were explained to participants, and an informed consent was signed by one of the parents or legal guardians (at 13 years, it was also signed by the participants). The baseline evaluation was additionally approved by the Data Protection National Commission, and the study follows the present EU General Data Protection Regulation under close supervision of the Data Protection Office of ISPUP.

For IAN-AF 2015-2016, ethical approval was obtained from the National Commission for Data Protection, the Ethical Committee of the Institute of Public Health of the University of Porto and the Ethical Commissions of the Regional Administrations of Health. All participants provided written informed consent. For adult participants, this was obtained directly. For children and adolescents under

18, consent was secured from their parents or legal representatives. Additionally, adolescents aged 10 to 17 were required to provide their own independent written consent. All documents with identification data were treated separately and stored in a different dataset. The databases with individual information are anonymised, allowing the non-identification of the participants. The Data Protection Officer at the Institute of Public Health of the University of Porto ensured data protection.

2.1. The Generation XXI population-based birth cohort

2.1.1. Study design and participants

GXXI is an ongoing prospective population-based birth cohort assembled between April 2005 and August 2006 at the five public units providing obstetrical and neonatal care in the metropolitan area of Porto, Portugal (131). The eligible criteria to integrate the cohort study were mothers living in one of the six municipalities of the metropolitan area of Porto (Figure 2.1.), delivering at the public hospitals covering those municipalities, and giving birth to live babies with gestational age >24 weeks. In the 24 to 72 hours after delivery, all mothers were invited to participate in the cohort GXXI, and 91% accepted to enrol, resulting in 8,495 mothers and 8,647 children.

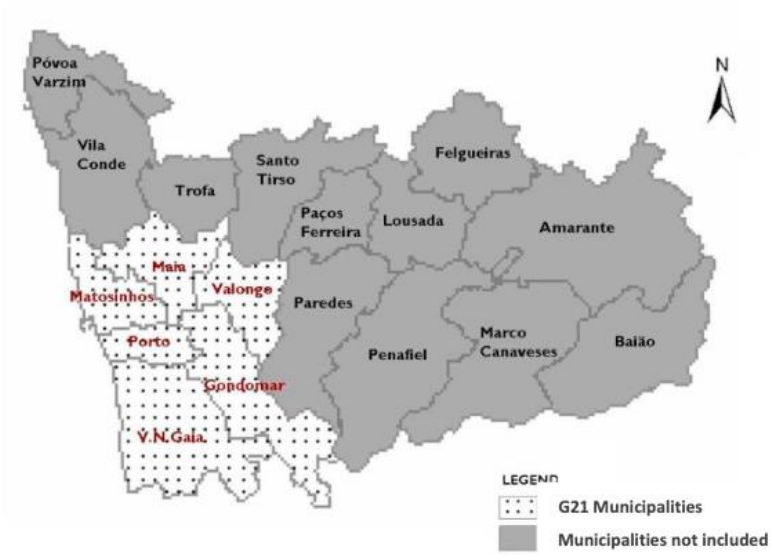

Figure 2.1. *Porto Municipalities included in the GXXI study.*

GXXI was the first large prospective population-based birth cohort study designed and implemented in Portugal, aimed to describe prenatal and postnatal development and to identify the associated factors. The collected information allowed the expansion and improvement of knowledge regarding the growth and development of children and adolescents throughout different life stages.

After baseline recruitment, mothers and children/adolescents have been invited to attend in-person interviews at the Department of Public Health and Forensic Sciences, and Medical School, Faculty of Medicine, University of Porto, Porto, Portugal. Subsamples were evaluated at 6 (n=1555), 15 (n=1043) and 24 months (n=855). Subsequently, all participants were invited to participate in the 4- (2009-2011), 7- (2012-2014), 10- (2015-2017) and 13-year follow-ups (2018-2020). The participation rates of each wave were 86%, 80%, 76% and 54%, respectively. The lower participation rate in the 13-year follow-up evaluation was related to the COVID-19 pandemic, prematurely interrupted in March 2020. Currently, the 18-year follow-up evaluation is ongoing.

This thesis uses data collected from the 4-year to 13-year-old evaluations in the GXXI cohort.

2.1.2. Data collection

Trained interviewers applied structured questionnaires in a face-to-face interview to collect information from mothers and children regarding sociodemographic characteristics, medical history, lifestyle factors, and dietary intake at baseline and all follow-up evaluations. During follow-up evaluations, trained nurses conducted clinical examinations, measured anthropometric parameters and blood pressure, and collected blood and urine samples.

Sociodemographic and maternal characteristics

At baseline, trained researchers inquired mothers about their socioeconomic conditions, medical history, pre-pregnancy and prenatal care, food habits during pregnancy, and maternal exposures before and during pregnancy. Researchers

also performed a physical examination. After that, at the 4-year follow-up, mothers were asked about their smoking habits (current smokers, former smokers or never smoked). For the purpose of this thesis, the maternal sociodemographic characteristics included were maternal age at baseline (in years), educational level at baseline (number of completed schooling years), smoking habits at 4-year follow-up (categorised in yes, current smokers, or no, former smokers or never smoked). Moreover, the sex of the child, collected at baseline, was also included.

Child and adolescents' anthropometric and blood pressure measurements

At 4, 7, 10, and 13-year follow-ups, body weight and height were measured by trained professionals under standard procedures, after 12 hours of fasting and with participants in light clothing and barefoot position (134). Using a wall stadiometer (SECA, Hamburg, Germany) and a digital scale (Tanita®, Arlington Heights, Illinois, US, model TBF 300), the height was measured to the nearest 0.1 centimetre and body weight to the nearest 0.1 kilogram, respectively. The body mass index (BMI) was calculated as weight over the squared height and computed according to age and sex-specific BMI standard deviation scores (z-BMI) published by the World Health Organization (WHO) (135).

Tetrapolar bioelectric impedance analysis was performed using BIA 101 Anniversary® (Akern, Florence, Italy), according to standard procedures (136). The percentage of fat mass (%FM) was derived from the Schaefer et al. equation (137).

Waist circumference (WC) was measured at the umbilicus level to the nearest 0.1 centimetre, with the participant in a standing position, relaxed abdomen, arms at the sides and feet together (134,138).

At 4- and 7-year follow-ups, after a 10-minute rest, with an interval of at least 5 minutes, using an automatic upper arm blood pressure monitor (Omron®) placed over the brachial artery pulse, two measurements of systolic (SBP) and diastolic (DBP) blood pressure were taken, and their mean value calculated. If the difference between the SBP and DBP measurements was >5 mmHg, a third

measurement was taken and considered the mean of the two closest values. At the 10- and 13-year follow-ups, three measurements of SBP and DBP were taken, and the mean of the two closest values was calculated. Age-, sex- and height-specific z-scores for SBP and DBP were calculated according to the American Academy of Pediatrics (139).

Child and adolescents' blood and urine samples

On the morning of all follow-up evaluations, after a 12-hour overnight fast, a blood sample was collected for all participants who had provided consent as well as their parents/main caregivers. Blood biomarkers laboratory analyses were performed at the Clinical Pathology Department of the Hospital of São João, Porto, Portugal.

At the 13-year follow-up, a convenience sample of 240 participants was invited to collect urine for 24 hours, as close as possible to the interview. All procedures were explained, orally and by writing, to adolescents and respective parents/main caregivers, and the necessary material was made available. Urinary creatinine was measured using the Jaffe method (Beckman Coulter) for all samples. Total BPA urinary concentration was measured using a dispersive liquid-liquid microextraction followed by gas chromatography coupled to mass spectrometry, considering 0.03 ng/mL and 0.1 ng/mL as the limit of detection (LOD) and quantification (LOQ), respectively (140).

Pubertal development

Pubertal development was assessed at ages 10 and 13 using the Tanner scale, a standardised method for evaluating sexual maturation (141,142). The scale evaluates various parameters, including breast development in girls, genital development in boys, and pubic hair development in both sexes. Additionally, axillary hair was assessed in both girls and boys at the age of 13. Trained professionals, previously instructed by a single endocrinologist, performed the evaluations. For girls, breast development was examined through both visual inspection and palpation, while genital development in boys was determined by

measuring testicular volume using the Prader orchidometer. Each parameter was categorised into one of five stages: stage 1 representing prepubertal, stages 2 through 4 indicating pubertal progression, and stage 5 postpubertal. In cases where breast or testicular development differed from pubic hair staging, the former was prioritised.

Child and adolescents' dietary intake

For the dietary intake assessment, parents or primary caregivers were invited to complete a three days food diary (including two weekdays and one weekend day) at the 4-, 7-, and 10-year follow-ups, while adolescents were invited to do the same at the 13-year follow-up. They received oral and written instructions from the research team before the face-to-face interview. The participants provided detailed information about each food and beverage consumed: consumption place and time, food preparation method, brand, quantity consumed, and packaging materials (only for 13-year follow-up). Trained researchers reviewed the food diaries during the face-to-face interview. They were codified a posteriori by a team of trained nutritionists into the Food Processor SQL software (143) at 4-, 7- and 10-year follow-ups and through eAT24 Module at 13-year follow-up, a software developed in the aim of the IAN-AF 2015-2016 (132). The methodology of "eAT24" software will be deeply described in the next section. For the longitudinal studies included in this work, dietary information from 4-, 7- and 10-year follow-ups were subsequently harmonised in the "eAT24" software.

Other variables

Lifestyle factors were also included in the current thesis analysis. In this line, parents or the primary caregiver were asked about the usual practice of some scheduled and regular sports activity outside the school setting at 4-, 7- and 10-year follow-ups. Adolescents were similarly questioned during the 13-year follow-up assessment. The practice of regular leisure physical activity was then categorised as yes or no and considered an important confounder variable in the present study.

2.2. The National Food, Nutrition and Physical Activity Survey of the Portuguese Population

2.2.1. Study design and participants

The IAN-AF 2015-2016 is a cross-sectional study nationally representative of the general Portuguese population between 3 months and 84 years of age (132). The survey assessed different dimensions of the Portuguese population, namely sociodemographic data, anthropometric measurements, dietary intake, physical activity and sedentary behaviours. Participants were randomly selected from the National Health Registry in each of the seven Statistical Geographical Units of Portugal (NUTs II) by multistage sampling and weighed according to sex and age groups (Figure 2.2.). In the first step, sampling was based on the random selection of the primary health care units stratified by the seven NUTs II, weighted by the number of individuals registered in each unit. The second step of sampling was based on the random selection of registered individuals in each primary health care unit, according to sex and age groups, considering this selection independent of the regular attendance to the National Health System (132). According to the complex sampling design of the IAN-AF 2015-2016 survey, the results were representative of the Portuguese population since each sample participant represented a specific number of individuals in this population.

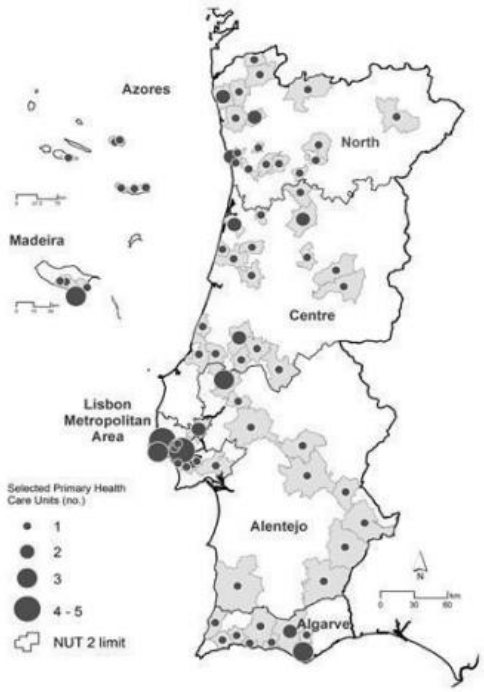

Figure 2.2. *Spatial distribution of the primary health care units, weighted by the number of registered individuals (NUT – Statistical Geographic Units of Portugal). Source: Lopes, 2018 (132).*

Consecutive recruitment waves ensured the use of the most recent versions of the National Health Registry lists in the sample selection. Exclusion criteria included residing in collective housing or institutions, living in Portugal for less than one year (except for infants), inability to speak Portuguese, physical or cognitive impairments that hindered participation (e.g., blindness, deafness, diagnosed dementia), or death.

From the 19 635 eligible participants, 6553 attempted the first interview, and 5811 completed two interviews. The participation rates were 33.4% and 29.6%, respectively, higher in children and adolescents and lower in adults and the elderly.

The first paper included in this thesis used data from the entire population (3 months to 84 years). However, the methods described in the present section and the discussion below will be focused on children and adolescent settings (n = 1959).

2.2.2. Data collection

Trained researchers (nutritionists or dietitians) conducted face-to-face interviews from October 2015 to September 2016, including all days of the week across the four seasons, and following European standard guidelines (144). The time interval between the first and second interviews was planned to be between 8 and 15 days. This survey was focused on the evaluation of nine dimensions: dietary and nutritional intake; eating habits and behaviours; dietary and nutritional supplements use; food insecurity; physical activity and sedentary behaviours; sociodemographic characteristics; general health data; anthropometrics; and, in a subsample, biochemical indicators of nutritional intake. Accordingly, the "You eAT&Move" software used for interviews was created and organised into three main modules to collect specific information:

1) You Module: sociodemographic information, anthropometric measurements, general health data, food insecurity, and food consumption patterns by a Food Propensity Questionnaire;
2) eAT24 Module: detailed food consumption data through 24-hour recalls or food diaries;
3) Move Module: physical activity data through the International Physical Activity Questionnaire, the Activity Choice Index, and Physical Activity diaries.

Children and adolescents' sociodemographic characteristics

The information on the participant's sex and age (calculated using the evaluation date and the participant's date of birth) was imported from the National Health Registry database. Parents or other primary caregivers completed the questionnaires for participants aged 9 years or younger. From 10 years old, all participants were invited to complete the questionnaires individually.

Regarding educational level, for children and adolescents, it was considered the highest number of completed years of schooling of one of the parents.

Finally, according to the participants' parish of residence and the classification of urban areas created by the Portuguese National Institute of Statistics (145), three

categories of geographical regions were defined: predominantly urban areas, medium urban areas and predominantly rural areas.

Child and adolescents' anthropometric measurements

Height (length for children younger than 2 years) and weight were measured objectively by trained observers according to standard procedures. Height was measured to the nearest centimetre using a portable wall stadiometer (SECA 213, Hamburg, Germany), with participants standing in light clothing and bare feet. For children under 2 years old, the recumbent length was measured with a measuring rod with large callipers to the nearest 0.1 cm (SECA 207; Hamburg, Germany). In the same conditions described above, body weight was measured using a digital scale (SECA 813, Hamburg, Germany) to the nearest 0.1 kg. For children under 2 years old, a specific paediatrics digital weight scale near 0.01 kg (SECA 354, Hamburg, Germany) was used with participants naked and without a diaper.

The BMI was calculated as weight over the squared height, and their categories were defined according to the WHO standards (underweight, normal weight, overweight and obesity). Age and sex-specific BMI z-scores were used for children and adolescents (135).

Child and adolescents' dietary intake

Dietary intake was obtained through two non-consecutive one-day food diaries for children under 10 or 24-hour recalls for the remaining sample (from 00h00 to 23h59). This information was collected using the eAT24 Module (Electronic Assessment Tool for 24-hour recall), a validated computerised tool to collect and process dietary data from 24-hour recall based on the automated multiple-pass method for 24-hour (5 steps) (132,146). All items, including beverages and dietary supplements, consumed during the previous 24 hours were recorded per eating occasion and eating place, quantified and described as eaten. This methodology requires describing all food items through several facets and respective descriptors using the FoodEx2 classification system (147). In this way,

it was possible to collect extensive information about participants' food intake, including food packaging format and materials (pottery, wood, cork, glass, paper, paperboard, vegetal paper, film, plastic, textiles, multilayer materials, metal, aluminium, edible materials and other materials). Finally, different methods were available for quantification of food items and recipes, such as weight and volume, photo series included in a food picture book (186 food photo series with six portions for each food or recipe and 11 photo series with household measures), standard units, household measures and default portion (148).

The final conversion of food data into energy and nutrients resulted from applying this validated "eAT24" module, which integrated the Portuguese Food Composition Table and European Food Information Resource (EuroFIR) databases for missing items.

Other variables

For participants between 3 and 17 years old, physical activity levels were assessed by asking about their regular practice in any scheduled sports or physical activities. Finally, the general questionnaire also gathered information on each participant's household composition. The practice of regular physical activity and the household composition were considered important variables in the present thesis.

RESULTS

Paper I

Risk characterisation of dietary acrylamide exposure and associated factors in the Portuguese population

Sofia Almeida Costa, Daniela Correia, Catarina Carvalho, Sofia Vilela, Milton Severo, Carla Lopes, Duarte Torres

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Risk characterization of dietary acrylamide exposure and associated factors in the Portuguese population

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ABSTRACT

Acrylamide exposure, mainly resulting from food cooking and processing, has been associated with a higher risk of health problems, due to genotoxic effects. This study aims to estimate acrylamide dietary exposure of the Portuguese population and its associated factors. Dietary data collected through 2 non-consecutive 24 hour recalls or food diaries from a representative sample of the Portuguese population from the National Food, Nutrition and Physical Activity Survey was used ($n = 5811$; 3–84 years). Occurrence data of acrylamide in food were obtained from EFSA. The margins of exposure (MOE) were calculated for peripheral neuropathy and neoplastic effects. The association between acrylamide and socio-demographic characteristics was estimated through linear regression models. For the total population, the estimated median daily dietary exposure per body weight to acrylamide was 0.38 μg/kg/day, ranging from 0.14 to 0.88 μg/kg/day for the 5th and 95th percentile, respectively. Children aged between 1–2 years had the highest acrylamide exposure (median 0.75 μg/kg/day, 95th percentile 1.41 μg/kg/day). For the peripheral neuropathy and neoplastic effects, the median MOE estimated was 1140 and 451, respectively. Men compared to women had a higher acrylamide dietary exposure, as well as smokers compared to non-smokers. Elderly and less educated individuals were inversely associated with acrylamide exposure. 'Bread and rusks' (24.2%) were the main source of acrylamide, followed by 'coffee' (21.3%). The current dietary exposure to acrylamide in the Portuguese population is of concern mainly regarding neoplastic effects. Our results point to the need to reduce exposure to acrylamide, especially in men, young children, higher educated individuals and smokers.

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Introduction

Diet is the fundamental basis to provide energy and essential nutrients for maintaining the body's homeostasis. However, these elements are often ingested with other compounds, considered as potentially hazardous to human health. They may result from environmental contamination or be generated non-intentionally, during production, processing, treatment, packaging, transport, storage, preparation and/or cooking. Acrylamide, heterocyclic amines, furan, acrolein and polycyclic aromatic hydrocarbon are some examples of hazardous contaminants produced during thermal treatments in addition to other desirable chemical and physical changes, responsible for flavours, aromas, colours and texture (Mottram et al. 2006).

Particularly, acrylamide is a compound formed through the Maillard reaction when the amino acid asparagine reacts with reducing sugars, such as glucose and fructose, at temperatures higher than 120 °C, under low moisture conditions (World Health Organization 2002; Zyzak et al. 2003; Murkovic & Pedreschi 2017). Therefore, the production of acrylamide is highly associated with industrial or home-cooking thermal methods like frying, baking, roasting and toasting (Murkovic & Pedreschi 2017).

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Foodstuffs rich in carbohydrates are more likely to produce high levels of acrylamide during their processing or cooking. Hence, cereal products (bread, toasts, breakfast cereals, biscuits and snacks), potato products (potato chips, French fries and crisps), coffee and coffee substitutes are the main sources of acrylamide through the human diet (Wenzl et al. 2007; EFSA CONTAM Panel 2015). Eating patterns and cooking habits also influence the exposure or the formation of acrylamide and, consequently, might have a different impact on health.

Besides diet, dermal absorption or inhalation are important routes when acrylamide exposure occurs in occupational settings or through tobacco smoke (Turkington & Mitchell 2009). In fact, acrylamide is present at high levels in cigarette smoke, or other tobacco products, and the European Food Safety Authority (EFSA) stated that this represents the most prominent source of acrylamide exposure in smokers and an important source to passive smokers (EFSA CONTAM Panel 2015). In the last few years, the interest in acrylamide's consequences to human health has been growing. Different organizations identified this contaminant as a public health concern due to its negative impact on the human organic system (International Agency for Research on Cancer 1994; EFSA CONTAM Panel 2015; European Commission 2017). In 1994, the International Agency for Research on Cancer classified acrylamide as probably carcinogenic (International Agency for Research on Cancer 1994) and, in 2015, the CONTAM Panel of EFSA defined a benchmark dose lower confidence limit for a 10% extra risk for peripheral neuropathy and neoplastic effects ($BMDL_{10}$ of 0.43 and 0.17 mg of acrylamide/kg of body weight/day, respectively) (EFSA CONTAM Panel 2015). Moreover, this scientific body concluded that there was a concern for neoplastic effects based on the margins of exposure (MOE) to acrylamide (EFSA CONTAM Panel 2015).

In Portugal, no information about individual exposure to acrylamide from dietary intake is available, and it becomes relevant to design new studies to understand the level of exposure in the population and its impact on the arising human disorders. Additionally, this knowledge will be useful to develop indicators that will help public health professionals and policymakers in the formulation of new and relevant health policies. Thus, the aim of this study was to estimate acrylamide dietary exposure of the Portuguese population and its associated factors, analysing the contribution of specific food groups to the usual exposure to acrylamide.

Material and methods

This study used data from the National Food, Nutrition and Physical Activity Survey 2015–2016 (IAN-AF 2015–2016) (Lopes et al. 2017; Lopes, Torres, Oliveira, Severo, Guiomar, et al. 2018). It consisted of a sample of people, aged between 3 months and 84 years old, randomized by a multistage sampling of the seven statistical geographic units of Portugal (NUTS II), weighed to the number of individuals registered in each health unit, sex and age groups.

The IAN-AF 2015–2016 assessed different dimensions of the Portuguese population, such as sociodemographic data, anthropometric measurements, dietary intake, physical activity and sedentary behaviours.

For this study, all participants with two valid dietary assessments were considered, resulting in a sample of 5811 individuals of which 52% were women, 66% were adults or elderly and 45% had the 3rd cycle or high educational level, as previously described (Lopes et al. 2017; Lopes, Torres, Oliveira, Severo, Guiomar, et al. 2018; Oliveira et al. 2018). The final sample of participants with less than 3 years old was 806, 1153 were children or adolescents (aged between 3 to 17 years) and 3852 were individuals of more than 18 years old (Lopes, Torres, Oliveira, Severo, Alarcão, et al. 2018).

Sociodemographic and general health data

Educational level was obtained by asking participants the number of completed years of schooling. For participants under 18 years old, it was considered the highest completed years of schooling of one of the parents. Three categories of the educational level were defined ('none, 1st

and 2nd cycle', '3rd cycle and high school' and 'higher education').

According to participants' parish of residence and the classification of urban areas created by the Portuguese National Institute of Statistics (Instituto Nacional de Estatistica (Portugal) 2014), three categories of geographical regions were defined, namely, predominantly urban areas (PUA), medium urban areas (MUA) and predominantly rural areas (PRA).

Participants' household composition was also collected, as well as the food insecurity status of the household, measured through an adapted Portuguese version of the questionnaire previously developed (Radimer et al. 1990; Alarcão et al. 2020). Among participants aged between 3 and 17 years, physical activity level was measured by the usual practice of asking about scheduled and regular sports activity. Adults were classified into inactive, minimally active or very active categories by applying the short-form International Physical Activity Questionnaire (SFIPAQ) (Craig et al. 2003; IPAQ Research Committee 2005).

Finally, individuals were asked about their smoking habits: smokers (currently or in the past) or non-smokers. For this study, data from adult smokers were analysed as a potentially associated factor to acrylamide exposure.

Anthropometrics measurements

Height (or length for children of less than 2 years old) and weight were objectively measured during the first interview, according to standard procedures (Guerra et al. 2014). For height measurements, to the nearest 1 cm a portable wall stadiometer was used (SECA 213, Hamburg, Germany), while for children under 2 years old, the recumbent length was measured to the nearest 0.1 cm with a measuring rod with large callipers (SECA 207; Hamburg, Germany). Body weight was measured using a digital scale (SECA 813, Hamburg, Germany) to the nearest 0.1 kg and for children under 2 years old, a paediatric digital weight scale approximates to 0.01 kg (SECA 354, Hamburg, Germany) was used (Oliveira et al. 2018).

Dietary assessment methods

Data on dietary intake was obtained by trained interviewers (dietitians or nutritionists), between October 2015 and September 2016, following European standard guidelines (European Food Safety Authority 2014). Parents of children aged less than 10 years old were asked to report two non-consecutive one-day food diaries, while the remaining participants reported two non-consecutive 24-hour recalls, with 8 - 15 d interval.

The collection and processing of food data were performed using the validated 'eAT24' software (Lopes, Torres, Oliveira, Severo, Guiomar, et al. 2018; Goios et al. 2020). During the interview, the participants were asked to report, quantify and describe all items consumed at each eating occasion, including beverages and dietary supplements. The FoodEx2 classification system was implemented allowing a complete description of all food items reported, through several facets and corresponding descriptors (European Food Safety Authority 2011). Thus, it was possible to collect a large set of information about the participants' food intake, including the place of food preparation-producing, cooking process and extent of cooking. For the quantification of food items and recipes, different methods were available, such as weight (in grams), volume (mL), food picture series (Torres et al. 2017), standard units, household measures and default portions.

Assessment of dietary acrylamide exposure

Data on the occurrence of acrylamide in food groups were extracted from the EFSA Scientific Opinion on Acrylamide in Food, published in 2015 (EFSA CONTAM Panel 2015). For each food group, the limit of quantification (LOQ), the percentage of left-censored samples (LC), the mean levels and the 95th percentile of acrylamide distribution reported by EFSA were used to estimate the acrylamide levels in the foods consumed by the Portuguese population. First, a FoodEx2 code (base term and process facet – F28) was attributed to each food category reported by EFSA (EFSA CONTAM Panel 2015). These FoodEx2 codes were used to merge the

food consumption data with the occurrence data. When more than one corresponding FoodEx2 code was available in the occurrence table (fried potatoes), one entry was randomly selected. After, for each food item reported in the IAN-AF 2015–2016 a value from the acrylamide distribution from the corresponding food group, published by EFSA, was randomly attributed: in 5% of the reports the 95th percentile level was attributed; LOQ values were attributed to a fraction of the reports corresponding to the LC reported (upper bound approach); for the other reports, the calculated true mean of the remaining values of acrylamide concentration was attributed, independently of the method used for handling LC data (Table S1). Finally, 10 cycles of random attributions were performed, which resulted in 10 different estimated values of acrylamide for each consumption occasion.

The margins of exposure (MOE) were calculated using the lower confidence limit of the benchmark dose for a 10% extra risk ($BMDL_{10}$) for peripheral neuropathy and neoplastic effects ($BMDL_{10}$ = 430 µg/kg of body weight/day and $BMDL_{10}$ = 170 µg/kg of body weight/day, respectively). For non-genotoxic outcomes, an MOE higher than 125 is considered sufficient to conclude that there is no health concern. For genotoxic and carcinogenic effects, a MOE higher than 10 000 presents a low level of concern for human health (EFSA CONTAM Panel 2015).

Ethics approval and consent to participate

The Survey IAN-AF 2015–2016 was approved by the National Commission for Data Protection, and ethical approval was obtained from the Ethical Committee of the Institute of Public Health of the University of Porto and the Ethical Commissions of each Regional Administrations of Health. All documents with identification data were treated separately and stored in a different dataset. All the participants gave their written informed consent according to the Ethical Principles for Medical Research involving human subjects expressed in the Declaration of Helsinki and the national legislation. Written agreements from the parents or legal representatives were required for children and adolescents under 18 years old. In addition, adolescents (10–17 years old) were also asked to provide independent written consent.

Statistics

All estimates were weighted for the Portuguese population distribution, according to the sampling design of the IAN-AF 2015–2016. The usual daily dietary exposure of acrylamide of the Portuguese population, in µg/kg of body weight, considering the dietary information from the two-days assessment and adjustment for the intra-individual variability, was calculated using SPADE software (1-Part model for daily-consumed foods or food components) (Dekkers et al. 2014) and analysed by sex, age and geographical region type. This methodology transforms the 2-day intake distribution of acrylamide into a normal distribution, using a Box-Cox transformation and models it as a function of age, estimating all model parameters including the variances within and between-participants. Then, a shrunken distribution is obtained by eliminating the within-person variance. Finally, the distribution is back-transformed to its original units. This analysis was normalized weighting for the complex survey design and the results are presented by percentiles of the distribution.

To test the association between acrylamide and socio-demographic characteristics, relative mean differences (RD) in the two-days mean exposure to acrylamide and the respective 95% confidence interval (CI) were estimated through linear regression models fit, after log-transforming the outcome due to its heavy-tailed distribution. The analysis was performed independently for children/adolescents and adults. For children and adolescents, the characteristics tested were sex, age categories, parents' educational level, geographical region type, household size and physical activity. For the adult population, it was analysed by sex, age groups, educational level, geographical region type, household size, IPAQ level, smoking habits and food insecurity. Three separate models were applied: a crude model; a model adjusted for sex, age groups and educational level (or parent's educational level for participants under 18 years old) (model 1); and,

exclusively in the adult population, a model adjusted for the same variables included in model 1, plus smoking habits (model 2).

The food groups that most contributed to the acrylamide exposure were identified for all the Portuguese population, and according to sex and age categories. The contribution to acrylamide exposure by the different food sources was expressed in percentage (%) and 95% CI. This analysis was weighted for the complex survey design.

All statistical analyses were performed separately for each one of the 10 different estimated values of acrylamide and combined using Rubin's rules (Marshall et al. 2009). A significance level of 5% was assumed. Analyses were performed using the library 'survey' of R software (The R Project for Statistical Computing) version 3.4.0 for Windows (Lumley 2020; R Core Team 2020).

Results

The overall median of usual dietary daily exposure to acrylamide in the Portuguese population was 0.38 µg/kg of body weight, ranging from 0.14 to 0.88 µg/kg of body weight (5th and 95th percentile, respectively) (Table 1). In this study, the estimations of acrylamide exposure did not differ according to sex and geographical region. However, some variation was observed between age groups. The daily median ranged from 0.29 µg/kg (95th percentile: 0.64 µg/kg) in children less than one year of age to 0.75 µg/kg (95th percentile: 1.41 µg/kg) in children aged 1–2 years. Globally from 3 years of age, the median value of acrylamide daily exposure decreased with increasing age, being 0.69, 0.54, 0.37 and 0.29 µg/kg, in the age groups 3–9 years, 10–17 years, 18–64 years and 65–84 years, respectively.

Table 1 also expresses the MOE values for peripheral neuropathy and neoplastic effects resulting from acrylamide exposure. For the peripheral neuropathy outcome, the Portuguese population MOE estimated for the median and the 95th percentile of acrylamide exposure was 1140 and 489, respectively. Despite these values did not differ between sex and geographical region categories, some differences were observed according to age. The youngest participants (1 to 17 years old) showed lower MOE values, especially those aged between 1 and 2 years who had an MOE of 571 for median and 304 for 95th percentile of exposure. According to the current acrylamide exposure, all MOE values were clearly

Table 1. The usual dietary daily dietary exposure to acrylamide (µg/kg of body weight/day) and margins of exposure values for peripheral neuropathy and neoplastic effects of acrylamide exposure, by sex, age group and geographical region type, and weighted for the complex survey design.

	Mean	P5[a]	P25[a]	Median	P75[a]	P95[a]
Total						
µg/kg/day	0.43	0.14	0.26	0.38	0.54	0.88
MOE_{PN}	1009	489	795	1140	1672	3060
MOE_{NE}	399	193	314	451	661	1210
Sex						
Female						
µg/kg/day	0.40	0.13	0.24	0.35	0.51	0.85
MOE_{PN}	1076	507	849	1234	1829	3389
MOE_{NE}	425	201	336	488	723	1340
Male						
µg/kg/day	0.46	0.15	0.28	0.41	0.58	0.93
MOE_{PN}	939	461	740	1054	1536	2788
MOE_{NE}	371	182	293	417	607	1102
Age groups						
year						
µg/kg/day	0.32	0.11	0.20	0.29	0.41	0.64
MOE_{PN}	1331	674	1048	1469	2121	3836
MOE_{NE}	526	267	414	581	839	1517
1–2 years						
µg/kg/day	0.80	0.36	0.56	0.75	0.99	1.41
MOE_{PN}	536	304	436	571	762	1202
MOE_{NE}	212	120	172	226	301	475
3–9 years						
µg/kg/day	0.74	0.32	0.51	0.69	0.91	1.32
MOE_{PN}	585	327	473	625	844	1357
MOE_{NE}	231	129	187	247	334	537
10–17 years						
µg/kg/day	0.58	0.24	0.40	0.54	0.73	1.07
MOE_{PN}	736	401	591	793	1087	1797
MOE_{NE}	291	159	234	313	430	710
18–64 years						
µg/kg/day	0.40	0.14	0.26	0.37	0.51	0.79
MOE_{PN}	1065	545	842	1172	1679	2991
MOE_{NE}	421	215	333	463	664	1182
65–84 years						
µg/kg/day	0.32	0.11	0.20	0.29	0.41	0.63
MOE_{PN}	1342	679	1056	1482	2143	3883
MOE_{NE}	530	268	418	586	847	1535
Geographical region type						
PUA						
µg/kg/day	0.43	0.14	0.26	0.38	0.54	0.88
MOE_{PN}	1003	487	792	1133	1656	3020
MOE_{NE}	396	192	313	448	655	1194
MUA						
µg/kg/day	0.43	0.14	0.26	0.38	0.54	0.88
MOE_{PN}	1010	489	797	1143	1672	3018
MOE_{NE}	399	193	315	452	661	1193
PRA						
µg/kg/day	0.41	0.13	0.24	0.36	0.52	0.89
MOE_{PN}	1041	484	824	1207	1790	3259
MOE_{NE}	411	191	326	477	708	1288

MOE: margins of acrylamide exposure; PUA: predominantly urban areas; MUA: medium urban areas; PRA: predominantly rural areas; P_{α}: α-percentile.
MOE for peripheral neuropathy ($BMDL_{10}$ = 430 µg/kg/day) (MOE_{PN}) and for neoplastic effects ($BMDL_{10}$ = 170 µg/kg/day) (MOE_{NE}).
[a]$P_{\alpha\ MOE} = BMDL_{10} / P_{1\ -\ \alpha\ exposure}$.

above 125, which indicates no public health concern regarding neurotoxicity.

Concerning the neoplastic effects on the Portuguese population, the MOE obtained for median and 95th percentile of exposure was 451 and 193, respectively. Differences between age groups were observed, where MOE increased with increasing age. The youngest participants presented the lowest MOE (226 for the median of exposure and 120 for the 95th percentile of exposure) compared to the general population. All estimated MOE for the neoplastic outcome were clearly under 10000, especially for children and adolescents, which indicate a public health concern for the Portuguese population.

Table 2 shows the association between the usual daily dietary exposure to acrylamide and socio-demographic characteristics among children and adolescents. It was observed that there was an association between acrylamide dietary exposure and age after the adjustment for sex, age groups and parent's educational level (model 1). Children of less than 1 year old had a 42% lower dietary exposure when compared to adolescents (10–17 years) (RD = 0.58 [95%CI: 0.42, 0.78]), while children aged 1 to 2 years and 3 to 9 years had 41% and 59% higher dietary exposure than adolescents, respectively (RD = 1.41 [95%CI: 1.23, 1.61]; RD = 1.59 [95%CI: 1.40, 1.81]). In adults, after adjustment, an association to the acrylamide dietary exposure was found for individuals of more than 65 years old and less educated (model 2) (RD = 0.84 [95%CI: 0.77, 0.91]; RD = 0.72 [95%CI: 0.65, 0.79], respectively) (Table 3). Moreover, men, compared to women, had an 18% higher dietary exposure to acrylamide, as well as smokers who showed a 13% higher dietary exposure compared to non-smokers (RD = 1.18 [95%CI: 1.10, 1.26]; RD = 1.13 [95%CI: 1.04, 1.24]). We observed associations between dietary exposure to acrylamide and household size and food insecurity in the crude model, which were dissolved after the adjustment to model 1.

The most relevant dietary contributors to acrylamide exposure, stratified by sex and age group are present in Table 4. The results showed that the food group 'bread and rusks' was the

Table 2. Associations between socio-demographic characteristics and the usual daily dietary exposure to acrylamide (μg/kg of body weight/day) among children and adolescents ($n = 1959$), weighted for the complex survey design.

	n	Crude model RD (95% CI)	Model 1 RD (95% CI)
Sex			
Female	986	ref	ref
Male	973	1.01 (0.92, 1.12)	1.03 (0.93, 1.13)
Age groups			
<1 year	238	**0.59 (0.43, 0.80)**	**0.58 (0.42, 0.78)**
1–2 years	568	**1.43 (1.25, 1.65)**	**1.41 (1.23, 1.61)**
3–9 years	521	**1.61 (1.42, 1.82)**	**1.59 (1.40, 1.81)**
10–17 years	632	ref	ref
Parents' educational level			
None, 1st and 2nd cycle	188	**0.78 (0.63, 0.97)**	0.85 (0.70, 1.03)
3rd cycle and high school	958	0.92 (0.83, 1.03)	0.95 (0.86, 1.05)
Higher education	799	ref	ref
Geographical region type			
PUA	1395	ref	ref
MUA	333	0.97 (0.85, 1.10)	0.97 (0.85, 1.11)
PRA	231	1.03 (0.90, 1.18)	1.06 (0.92, 1.21)
Household size			
2–3 members	703	ref	ref
4 members	486	0.99 (0.87, 1.13)	0.97 (0.85, 1.09)
≥ 5 members	254	0.89 (0.73, 1.08)	0.90 (0.74, 1.10)
Physical activity (yes/no)[a]			
Yes	457	1.01 (0.89, 1.15)	0.96 (0.85, 1.08)
No	674	ref	ref

RD: relative mean differences; CI: confidence interval; ref: reference category; PUA: predominantly urban areas; MUA: medium urban areas; PRA: predominantly rural areas.
Crude model – without adjustment; Model 1 – adjusted for sex, age groups and parent's educational level.
[a]Physical activity variable only for participants aged ≥3 years ($n = 1153$).
Significant values are in **bold**.

Table 3. Associations between socio-demographic characteristics and the usual daily dietary exposure to acrylamide (μg/kg of body weight/day) among adults ($n = 3852$), weighted for the complex survey design.

	n	Crude model RD (95% CI)	Model 1 RD (95% CI)	Model 2 RD (95% CI)
Sex				
Female	2032	ref	ref	ref
Male	1820	**1.19 (1.11, 1.27)**	**1.18 (1.10, 1.26)**	**1.17 (1.09, 1.25)**
Age groups				
18–64 years	3102	ref	ref	ref
65–84 years	750	**0.72 (0.67, 0.78)**	**0.84 (0.77, 0.91)**	**0.85 (0.78, 0.93)**
Educational level				
None, 1st and 2nd cycle	1342	**0.67 (0.61, 0.74)**	**0.72 (0.65, 0.79)**	**0.72 (0.65, 0.79)**
3rd cycle and high school	1629	0.97 (0.90, 1.04)	0.96 (0.89, 1.03)	0.95 (0.89, 1.03)
Higher education	876	ref	ref	ref
Geographical region type				
PUA	2803	ref	ref	ref
MUA	680	0.96 (0.87, 1.06)	0.99 (0.90, 1.09)	0.99 (0.90, 1.08)
PRA	369	0.95 (0.80, 1.11)	0.99 (0.85, 1.15)	0.98 (0.85, 1.14)
Household size				
1–2 members	1588	ref	ref	ref
3–4 members	1898	**1.18 (1.12, 1.26)**	1.03 (0.96, 1.11)	1.04 (0.97, 1.12)
≥ 5 members	320	**1.24 (1.08, 1.43)**	1.13 (0.97, 1.31)	1.13 (0.98, 1.31)
IPAQ level				
Inactive	1623	ref	ref	ref
Minimally active	1176	0.99 (0.91, 1.07)	0.98 (0.91, 1.06)	0.98 (0.91, 1.06)
Very active	937	1.05 (0.96, 1.15)	1.03 (0.94, 1.12)	1.03 (0.94, 1.12)
Smoking habits				
Smokers	812	**1.24 (1.14, 1.34)**	**1.13 (1.04, 1.24)**	**1.13 (1.04, 1.24)**
Non-smokers	3037	ref	ref	ref
Food insecurity				
Yes	397	**0.81 (0.72, 0.91)**	0.89 (0.79, 1.01)	0.89 (0.79, 1.01)
No	3448	ref	ref	ref

RD: relative mean differences; CI: confidence interval; ref: reference category; PUA: predominantly urban areas; MUA: medium urban areas; PRA: predominantly rural areas; IPAQ: International Physical Activity Questionnaire.
Crude model – without adjustment; Model 1 – adjusted for sex, age groups and educational level; Model 2 – adjusted for model 1 and smoking habits.
Significant values are in **bold**.

highest contributor to acrylamide exposure in all population groups. In relation to sex, a higher contribution to acrylamide exposure of 'coffee' and 'cookies and biscuits' was observed in females when compared to males (22.5% vs 19.9% and 12.8% vs 9.7%, respectively). Some differences between adults and the elderly were observed in the dietary contributors to acrylamide, especially for the 'bread and rusks', 'coffee' and 'potatoes and other starchy tubers' groups. For the elderly (65–84 years) and compared to adults (18–64 years), the contribution of bread and coffee was higher (33.1% vs 22.6% and 28.3% vs 23.9%, respectively), while the contribution to acrylamide exposure from potatoes and other starchy tubers' was lower (8.4% vs 15.4%).

Children and adolescents showed different contributions to acrylamide exposure compared to other age groups, expected due to the dietary patterns adopted during their lifetimes. For infants (<1 year), 'infant cereals', 'cookies and biscuits' and 'infant formulas' together represented 82.6% of the total acrylamide exposure, and in toddlers (1–2 years) 'infant cereals' and 'cookies and biscuits' were responsible for 45.6% of the total daily exposure to acrylamide. The main contributors to the total exposure of children aged between 3–9 years were 'bread and rusks' (22.6%), 'cookies and biscuits' (19.3%), 'potatoes and other starchy tubers' (16.4%) and 'breakfast cereals and cereal bars' (12.3%). In adolescents, the food groups most relevant to acrylamide exposure were 'bread and rusks' (21.9%) followed by 'potatoes and other starchy tubers' (18.0%), 'cookies and biscuits' (15.1%) and 'breakfast cereals and cereal bars' (14.4%).

Finally, the associations between the usual daily exposure to acrylamide and major food groups (Tables S2 and S3) were also analysed. Briefly, a higher consumption of bread, cakes, biscuits, salty snacks, breakfast cereals and starchy tubers was associated with a higher acrylamide exposure.

Table 4. Food sources that contribute to acrylamide exposure in percentage (95% CI), weighted for the complex survey design, by sex and age group.

Food groups	Total	Female	Male	<1 year	1–2 years	3–9 years	10–17 years	18–64 years	65–84 years
Bread and rusks	24.2 (23.7, 24.7)	24.6 (23.9, 25.2)	23.8 (23.1, 24.6)	4.9 (3.4, 6.4)	14.0 (12.6, 15.4)	22.6 (21.2, 23.9)	21.9 (20.6, 23.1)	22.6 (22.0, 23.2)	33.1 (31.6, 34.6)
Coffee	21.3 (20.8, 21.7)	22.5 (21.9, 23.1)	19.9 (19.2, 20.7)	–	0.2 (0.1, 0.3)	0.8 (0.5, 1.0)	1.9 (1.6, 2.2)	23.9 (23.3, 24.6)	28.3 (27.1, 29.6)
Potatoes and other starchy tubers	14.3 (13.7, 14.9)	12.9 (12.1, 13.7)	15.8 (15.0, 16.5)	0.4 (0.1, 0.7)	7.4 (6.2, 8.5)	16.4 (14.7, 18.1)	18.0 (16.3, 19.7)	15.4 (14.5, 16.3)	8.4 (7.3, 9.5)
Cookies and biscuits	11.3 (10.9, 11.7)	12.8 (12.1, 13.5)	9.7 (9.2, 10.2)	27.2 (23.3, 31.0)	29.2 (27.2, 31.2)	19.3 (17.8, 20.7)	15.1 (13.9, 16.3)	10.1 (9.6, 10.6)	9.9 (8.9, 11.0)
Breakfast cereals and cereal bars	6.0 (5.7, 6.2)	6.3 (5.9, 6.8)	5.5 (5.2, 5.9)	0.4 (0.1, 0.7)	8.1 (6.9, 9.3)	12.3 (11.2, 13.4)	14.4 (13.3, 15.4)	5.1 (4.7, 5.4)	3.5 (2.8, 4.1)
Cakes	5.0 (4.8, 5.3)	5.4 (5.0, 5.8)	4.6 (4.3, 4.9)	0.2 (0.0, 0.5)	2.0 (1.6, 2.5)	7.9 (6.9, 9.0)	7.5 (6.6, 8.4)	4.8 (4.5, 5.0)	4.2 (3.6, 4.8)
Savoury pies and finger foods	3.3 (3.0, 3.5)	3.3 (3.0, 3.7)	3.2 (2.8, 3.5)	–	1.4 (0.7, 2.2)	3.2 (2.5, 3.8)	5.5 (4.7, 6.2)	3.3 (3.0, 3.5)	2.4 (1.7, 3.1)
Nuts, oilseeds and processed products	3.2 (3.0, 3.3)	3.1 (2.8, 3.3)	3.3 (3.0, 3.5)	0.0 (0.0, 0.1)	1.3 (0.8, 1.7)	1.8 (1.4, 2.2)	2.0 (1.7, 2.3)	3.5 (3.2, 3.7)	3.2 (2.7, 3.7)
Beer	2.8 (2.5, 3.0)	0.6 (0.5, 0.7)	5.1 (4.6, 5.6)	–	–	0.1 (0.0, 0.2)	0.1 (0.1, 0.2)	3.7 (3.3, 4.0)	1.8 (1.4, 2.1)
Flour, bread dough and pastry dough	2.7 (2.5, 2.9)	2.6 (2.4, 2.8)	2.8 (2.5, 3.0)	0.9 (0.4, 1.4)	1.9 (1.4, 2.5)	2.5 (2.1, 3.0)	3.0 (2.6, 3.4)	2.8 (2.6, 3.0)	2.2 (1.9, 2.6)
Snacks and chips	2.0 (1.8, 2.2)	2.0 (1.7, 2.3)	2.0 (1.8, 2.2)	0.7 (0.0, 1.3)	2.0 (1.0, 3.0)	4.1 (3.3, 4.9)	4.3 (3.4, 5.1)	1.8 (1.6, 2.0)	1.0 (0.7, 1.3)
Pasta	1.7 (1.6, 1.8)	1.6 (1.5, 1.8)	1.8 (1.7, 1.9)	2.2 (1.4, 3.0)	4.3 (3.6, 4.9)	2.6 (2.4, 2.9)	2.6 (2.3, 3.0)	1.6 (1.5, 1.7)	1.2 (0.9, 1.4)
Sweets	1.1 (1.0, 1.1)	1.0 (0.9, 1.1)	1.1 (1.1, 1.2)	–	0.7 (0.5, 0.9)	2.6 (2.4, 2.9)	2.4 (2.1, 2.7)	0.9 (0.9, 1.0)	0.6 (0.5, 0.8)
Infant cereals	0.5 (0.4, 0.6)	0.4 (0.4, 0.5)	0.6 (0.5, 0.7)	35.0 (31.0, 39.1)	16.4 (14.7, 18.1)	2.1 (1.5, 2.6)	0.2 (0.1, 0.3)	0.1 (0.0, 0.1)	0.0 (0.0, 0.1)
Fresh fruit and fruit jars	0.2 (0.1, 0.2)	0.2 (0.1, 0.3)	0.1 (0.1, 0.1)	7.5 (5.3, 9.7)	6.2 (5.0, 7.4)	0.3 (0.2, 0.5)	0.5 (0.0, 1.0)	–	–
Infant formula	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	20.4 (17.4, 23.4)	3.4 (2.7, 4.2)	–	–	–	–

CI: confidence interval.

Discussion

The present study estimated the dietary exposure to acrylamide by the Portuguese population. For the total population, the estimated median dietary exposure to acrylamide was 0.38 µg/kg/day, while children and adolescents were the groups with higher acrylamide exposure and higher health concern regarding neoplastic effects. The comparison of these results with other studies is difficult due to the considerable differences observed between dietary assessment methodologies and acrylamide occurrence databases used. Despite some methodological differences, the results observed were in accordance with other studies and reports (EFSA CONTAM Panel 2015; Food Safety Authority of Ireland 2016; Abt et al. 2019). Data collected within National Health and Nutrition Examination Survey (NHANES), estimated a mean dietary exposure in the United States population of 0.36 µg/kg of body weight/day, with the 90th percentile of 0.86 µg/kg of body weight/day (Abt et al. 2019). The EPIC study, involving 10 European countries, found that women presented a substantially lower acrylamide intake compared to men, as also observed by other authors (Freisling et al. 2013; Zając et al. 2013; Wyka et al. 2015). A French infant study concluded that the mean estimated for acrylamide exposure in children aged between 12 to 36 months was 0.74 µg/kg of body weight/day, with the 90th percentile of 1.66 µg/kg of body weight/day (Sirot et al. 2019). Also, another study conducted in the Irish children and adult population showed an acrylamide dietary exposure similar to the present results (mean of 0.71 and 0.38 µg/kg of body weight/day; and 97.5th percentile of 1.42 and 1.03 µg/kg of body weight/day, respectively) (Food Safety Authority of Ireland 2016).

Nevertheless, the acrylamide exposure calculated for the Portuguese population was lower than the estimates reported for other countries (Claeys et al. 2010; Freisling et al. 2013; Zając et al. 2013) and by EFSA (EFSA CONTAM Panel 2015). These differences can be explained by several factors. For example, compared to other European countries, the Portuguese population consume less quantity of foods, that especially

contribute to acrylamide exposure, namely bread, coffee, biscuits and cakes (Leclercq et al. 2009; Dubuisson et al. 2010; Irish Universities Nutrition Alliance 2011; De Ridder et al. 2016; Irish Universities Nutrition Alliance 2018; Lopes, Torres, Oliveira, Severo, Alarcão, et al. 2018; Irish Universities Nutrition Alliance 2021). Thus, it seems that food habits/patterns acquired or distinctive from a specific cultural context, have a strong effect on acrylamide exposure (Freisling et al. 2013). Also, it is important to note that in the Portuguese population there is a 29.9% misreporting prevalence on energy intake, from which, 28.5% is from under-reporting, which could explain some of the observed differences (Magalhães et al. 2020).

Neoplastic effects of human acrylamide exposure remain unclear, despite the carcinogenicity observed in rodents since the established association was with the Harderian gland, an organ not found in the human body. Even so, the CONTAM Panel of EFSA concluded that the MOE, across dietary surveys and age groups, indicate a concern regarding neoplastic effects, even with the inter-species differences taken into account and without the demonstration of carcinogenicity in humans through epidemiological studies (EFSA CONTAM Panel 2015). In the present study, MOE values were substantially below 10000, similar to other studies, and indicate a concern from a public health perspective for all groups of population (EFSA CONTAM Panel 2015; Food Safety Authority of Ireland 2016; Sirot et al. 2019; Van Bussel et al. 2020). However, because of their higher exposure per body weight and their immature system, children and adolescents represent a particularly susceptible population group, pointing to the need of reducing acrylamide exposure in these age groups.

Regarding the factors associated with acrylamide exposure, our findings suggest that, in adults, men compared to women had a higher dietary acrylamide exposure, as well as smokers compared to non-smokers. Elderly and less educated individuals were inversely associated with acrylamide exposure. Some of these associations were observed by Duke et al (2018) namely regarding sex, age groups and smoking habits. Concerning tobacco use, the association observed in the present study raises the concern around this population group, as these individuals are already highly exposed to acrylamide due to the higher concentration of this compound in tobacco smoke.

The main contributors identified for the Portuguese population, namely bread, coffee and potato products, are in line with previous observations in other countries (Freisling et al. 2013). Nevertheless, children and adolescents had different main food sources of acrylamide compared to adults. Besides, even among children of different ages, the food sources varied considerably. This is expected as result of the natural differences in the consumption patterns of the different age groups, reflecting several transitions in food habits for specific ages. For children of less than 3 years of age, infant cereals, cookies and infant formulas were responsible for most of the total daily exposure to acrylamide, while for those aged between 3 and 17 years, the most important food groups were bread, cookies and breakfast cereals. However, the interpretation of the food contribution to acrylamide exposure is not so linear. In some occasions, the higher contributor is a food product with a low concentration of acrylamide, but the principal food source in that specific age, such as the case of infant formulas. Despite this, these results were in accordance with other authors, increasing the need to put pressure on industry to reduce the acrylamide content in the most contaminated food products or to change intake patterns in these population groups, promoting the choice of foodstuffs with lower levels of acrylamide, such as fruit and vegetables (Abt et al. 2019).

To reduce the presence of acrylamide in food, the European Commission (EC) published in 2017 a Regulation (EU 2017/2158) establishing mitigation measures and benchmark levels (European Commission 2017). The document states that levels of acrylamide should be as low as reasonably achievable and below the benchmark levels proposed, especially for certain foodstuffs as French fries or other products based on raw potatoes; potato crisps, snacks, crackers and other dough-based potato products; bread; breakfast cereals; fine bakery wares; coffee; coffee

substitutes; and baby food. A large number of mitigation measures are presented in the Regulation covering the entire food chain, namely measures applied to agronomy; raw material selection, storage and transportation; recipe; process design; and information to the final consumers (European Commission 2017). However, after the implementation of Regulation 2017/2158, in Portugal, the majority of the 540 analysed bread samples had an acrylamide concentration above the indicative values set by the EC (Jesus et al. 2018). This was also observed in other countries, such as Italy, and for a wide variety of food products (Branciari et al. 2020; Esposito et al. 2020).

Thus, the daily dietary acrylamide exposure is not only defined by its concentration, but also by the amount of food consumed. From the public health perspective, combined efforts should be taken to decrease the acrylamide levels in food and to change the dietary patterns by consumers, especially for the major contributors and mainly in children and adolescents (Duke et al. 2018; Sirot et al. 2019). Infant cereals, baby products, cookies and breakfast cereals were the most important dietary sources of acrylamide for these population groups and represent an opportunity to modify the food processing and/or the consumers' choices, reducing the exposure to this hazardous contaminant.

The strengths of this study were the data on food consumption obtained by a representative sample of the Portuguese population and the detail of the collected information, that followed a standardized European methodology (European Food Safety Authority 2014). The extension and quality of acrylamide data occurrence, in the same timeline of dietary intake information, also represents a strength of this study. Additionally, we included variability in the occurrence of acrylamide in foods using the information extracted from EFSA (EFSA CONTAM Panel 2015). Lastly, the usual daily intake was calculated using SPADE software (Dekkers et al. 2014), which allows the adjustment of intra-individual day-to-day variability, using survey weights assuring the national representativeness.

Data on acrylamide occurrence in food items were extracted from the EFSA report, which combines many analytical results, from different food associations and European countries. In this extensive publication, acrylamide levels are presented in three possible scenarios, that allowed the design of a methodology based on the attribution of occurrence levels to the different consumption events, based on the distribution of the original data, introducing variability on exposure estimations. However, the concentration of acrylamide in foodstuffs vary widely and is highly dependent on the ingredients composition and cooking processes, such as temperature and duration of heating (Jesus et al. 2018; Mesías et al. 2019; Esposito et al. 2020). In the current study, these differences, caused by the industry or during homemade preparations, were not considered, which may under (for those that over-cook or burn the food products) or overestimate the risk of acrylamide exposure (if the occurrence of acrylamide in food is lower than the one assigned), representing a limitation in the analysis. Conversely, the application of an upper bound approach for left-censored data may overestimate the acrylamide levels used as input to the exposure assessment. The fact that these results may not represent the current exposure, regarding the time prior to the implementation of the EC Regulation 2017/2158, represents another limitation. Even so, the extent to which industries and producers responded to this Regulation appears to be limited.

Overall, at present, there is a lack of information about individual exposure to acrylamide by the Portuguese population. This research represents the first step of the acrylamide risk assessment in Portugal and allows the design of future investigations to assess the potential cumulative effect of acrylamide exposure, as well as the combined exposure to different heat-generated compounds and their effects on health.

Conclusion

This study assessed and characterized the exposure to acrylamide, a xenobiotic derived from food processing that may have a negative impact on human health. To the best of our knowledge, this is the first study that estimated the dietary acrylamide exposure of the Portuguese

population, analysed the individual associated factors and identified the contribution of the main food groups. Our results confirm the concern regarding the current dietary exposure to acrylamide, mainly for neoplastic effects. Thus, this information can be used to design new and effective public health interventions, to reduce the exposure to acrylamide, especially in men, young children, most educated individuals and tobacco users.

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Author contributions

CL and DT contributed to the design and implementation of the research, DC and MS to the analysis of the results, SAC, DC and CC to the writing of the manuscript, SAC, DC, CC, SV, MS, CL and DT to the reviewing of the manuscript.

Disclosure statement

The authors report there are no competing interests to declare.

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References

Abt E, Robin LP, McGrath S, Srinivasan J, DiNovi M, Adachi Y, Chirtel S. 2019. Acrylamide levels and dietary exposure from foods in the United States, an update based on 2011-2015 data. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 36(10):1475–1490. doi:10.1080/19440049.2019.1637548.

Alarcão V, Guiomar S, Oliveira A, Severo M, Correia D, Torres D, Lopes C. 2020. Food insecurity and social determinants of health among immigrants and natives in Portugal. Food Sec. 12(3):579–589. doi:10.1007/s12571-019-01001-1.

Branciari R, Roila R, Ranucci D, Altissimi MS, Mercuri ML, Haouet NM. 2020. Estimation of acrylamide exposure in Italian schoolchildren consuming a canteen menu: health concern in three age groups. Int J Food Sci Nutr. 71(1):122–131. doi:10.1080/09637486.2019.1624692.

Claeys W, Baert K, Mestdagh F, Vercammen J, Daenens P, de Meulenaer B, Maghuin-Rogister G, Huyghebaert A. 2010. Assessment of the acrylamide intake of the Belgian population and the effect of mitigation strategies. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 27(9):1199–1207. doi:10.1080/19440049.2010.489577.

Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, et al. 2003. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 35(8):1381–1395. doi:10.1249/01.MSS.0000078924.61453.FB.

De Ridder K, Bel S, Brocatus L, Lebacq T, Ost C, Teppers E. 2016. Résumé des résultats. In: Tafforeau J éditor. Enquête de consommation alimentaire. Bruxelles: Dans. https://fcs.wiv-isp.be/nl/Gedeeldedocumenten/FRANS/Resume_FR_finaal_web.pdf

Dekkers AL, Verkaik-Kloosterman J, van Rossum CT, Ocké MC. 2014. SPADE, a new statistical program to estimate habitual dietary intake from multiple food sources and dietary supplements. J Nutr. 144(12):2083–2091. doi:10.3945/jn.114.191288.

Dubuisson C, Lioret S, Touvier M, Dufour A, Calamassi-Tran G, Volatier J-L, Lafay L. 2010. Trends in food and nutritional intakes of French adults from 1999 to 2007: results from the INCA surveys. Br J Nutr. 103(7):1035–1048. doi:10.1017/S0007114509992625.

Duke TJ, Ruestow PS, Marsh GM. 2018. The influence of demographic, physical, behavioral, and dietary factors on hemoglobin adduct levels of acrylamide and glycidamide in the general U.S. population. Crit Rev Food Sci Nutr. 58(5):700–710. doi:10.1080/10408398.2016.1215289.

EFSA CONTAM Panel. 2015. Scientific Opinion on acrylamide in food. EFSA J. 13(6):4104.

Esposito F, Velotto S, Rea T, Stasi T, Cirillo T. 2020. Occurrence of acrylamide in Italian baked products and dietary exposure assessment. Molecules. 25(18): 4156–4110. doi:10.3390/molecules25184156.

European Commission. 2017. Commission Regulation (EU) 2017/2158: establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. Off J Eur Union L. 304(315):24–44. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R2158&from=EN.

European Food Safety Authority. 2011. Report on the development of a Food Classification and Description System for exposure assessment and guidance on its implementation and use. Efsa J. 9(12):1–84.

European Food Safety Authority. 2014. Guidance on the EU Menu methodology. EFSA J. 12(12):3944.

Food Safety Authority of Ireland 2016. Report on a total diet study carried out by the Food Safety Authority of Ireland in the period 2012 – 2014. www.fsai.ie

Freisling H, Moskal A, Ferrari P, Nicolas G, Knaze V, Clavel-Chapelon F, Boutron-Ruault MC, Nailler L, Teucher B, Grote VA, et al. 2013. Dietary acrylamide intake of adults in the European Prospective Investigation into Cancer and Nutrition differs greatly according to geographical region. Eur J Nutr. 52(4): 1369–1380. doi:10.1007/s00394-012-0446-x.

Goios ACL, Severo M, Lloyd AJ, Magalhães VPL, Lopes C, Torres DPM. 2020. Validation of a new software eAT24 used to assess dietary intake in the adult Portuguese population. Public Health Nutr. 23(17):3093–3011. doi: 10.1017/S1368980020001044.

Guerra RS, Fonseca I, Pichel F, Restivo MT, Amaral TF. 2014. Hand length as an alternative measurement of height. Eur J Clin Nutr. 68(2):229–233. doi:10.1038/ejcn.2013.220.

Instituto Nacional de Estatistica (Portugal). 2014. The new Classification of urban areas. Deliberation of the Standing Section of Statistical Coordination of the Statistical Council. www.ine.pt

International Agency for Research on Cancer 1994. IARC monographs on the evaluation of carcinogenic risks to humans: some industrial chemicals. Lyon.

IPAQ Research Committee 2005. Guidelines for data processing and analysis of the International Physical Activity Questionnaire (IPAQ) - Short Form.

Irish Universities Nutrition Alliance 2011. National adult nutrition survey.

Irish Universities Nutrition Alliance 2018. National Children' s Food Survey II: main report [Internet]. https://irp-cdn.multiscreensite.com/46a7ad27/files/uploaded/NCFSIIMainSurveyReport.pdf

Irish Universities Nutrition Alliance 2021. National Teens' food survey II summary report [Internet]. https://irp.cdn-website.com/46a7ad27/files/uploaded/NTFSIISummaryReport2021.pdf

Jesus S, Delgado I, Rego A, Brandão C, dos Santos R, Bordado J, Castanheira I. 2018. Determination of Acrylamide in Portuguese Bread by UPLC-MS/MS: Metrological and Chemometric tools. Acta Imeko. 7(2): 96. doi:10.21014/acta_imeko.v7i2.453.

Leclercq C, INRAN-SCAI 2005-06 Study Group, Arcella D, Piccinelli R, Sette S, Le Donne C, Turrini A. 2009. The Italian National Food Consumption Survey INRAN-SCAI 2005-06: main results in terms of food consumption. Public Health Nutr. 12(12):2504–2532. doi:10.1017/S1368980009005035.

Lopes C, Torres D, Oliveira A, Severo M, Alarcão V, Guiomar S, Mota J, Teixeira P, Rodrigues S, Lobato L. 2018. National food, nutrition, and physical activity survey of the portuguese general population, IAN-AF 2015-2016: summary of results [Internet]. www.ian-af.up.pt

Lopes C, Torres D, Oliveira A, Severo M, Guiomar S, Alarcão V, Ramos E, Rodrigues S, Vilela S, Oliveira L, et al. 2018. National food, nutrition, and physical activity survey of the Portuguese general population (2015-2016): Protocol for design and development. J Med Internet Res. 20(2):1–11.

Lopes C, Torres D, Oliveira A, Severo M, Guiomar S, Alarcão V, Vilela S, Ramos E, Rodrigues S, Oliveira L, et al. 2017. National Food, Nutrition and Physical Activity Survey of the Portuguese general population. EFSA Support Publ. 14(12):e42.

Lumley T. 2020. Survey: analysis of complex survey samples. R package version 4.0.

Magalhães V, by IAN-AF Consortium, Severo M, Torres D, Ramos E, Lopes C. 2020. Characterizing energy intake misreporting and its effects on intake estimations, in the Portuguese adult population. Public Health Nutr. 23(6): 1031–1040. doi:10.1017/S1368980019002465.

Marshall A, Altman DG, Holder RL, Royston P. 2009. Combining estimates of interest in prognostic modelling studies after multiple imputation: current practice and guidelines. BMC Med Res Methodol. 9(1):57. doi:10.1186/1471-2288-9-57.

Mesías M, Morales FJ, Delgado-Andrade C. 2019. Acrylamide in biscuits commercialised in Spain: A view of the Spanish market from 2007 to 2019. Food Funct. 10(10):6624–6632. doi:10.1039/C9FO01554J.

Mottram DS, Low MY, Elmore JS. 2006. The Maillard reaction and its role in the formation of acrylamide and other potentially hazardous compounds in foods. In: Skog K, Alexander J, editors. Acrylamide other hazard compd heat-treated foods. Cambridge: Woodhead Publishing; p. 3–22. https://www.sciencedirect.com/science/article/pii/B978184569011350001X

Murkovic M, Pedreschi F. 2017. Potentially toxic food components formed by excessive heat processing. In: Uribarri J, editor. Diet AGEs their role heal dis. 1st ed. Boca Raton, FL: CRC Press; p. 87–101. doi:10.1201/9781315120041

Oliveira A, by the IAN-AF Consortium, Araújo J, Severo M, Correia D, Ramos E, Torres D, Lopes C, Rodrigues S, Vilela S, Guiomar S, et al. 2018. Prevalence of general and abdominal obesity in Portugal: Comprehensive results from the National Food, nutrition and physical activity survey 2015-2016. BMC Public Health. 18(1):1–9. www.ian-af.up.pt. doi:10.1186/s12889-018-5480-z.

R Core Team 2020. R: A language and environment for statistical computing [Internet]. Vienna: R Foundation for Statistical Computing. https://www.r-project.org/

Radimer KL, Olson CM, Campbell CC. 1990. Development of indicators to assess hunger. J Nutr. 120(Suppl_11): 1544–1548. doi:10.1093/jn/120.suppl_11.1544.

Sirot V, Rivière G, Leconte S, Vin K, Traore T, Jean J, Carne G, Gorecki S, Veyrand B, Marchand P, et al. 2019. French infant total diet study: Dietary exposure to heat-induced compounds (acrylamide, furan and polycyclic aromatic hydrocarbons) and associated health risks . Food Chem Toxicol. 130:308–316. doi:10.1016/j.fct.2019.05.009.

Torres D, Faria N, Sousa N, Soares R, Amorim H, Guiomar S, Lobato L, Oliveira C, Correia D, Carvalho C, et al. 2017. Manual Fotográfico de Quantificação de Alimentos [Internet]. Universidade do Porto, p. 2017. www.ian-af.up.pt

Turkington C, Mitchell D. 2009. The encyclopedia of poisons and atidots. 3rd ed. New York: Estate of Carol Turkington.

Van Bussel LM, Van Rossum CTM, Temme EHM, Boon PE, Ocké MC. 2020. Educational differences in healthy, environmentally sustainable and safe food consumption among adults in the Netherlands. Public Health Nutr. 23(12):2057–2067. doi:10.1017/S1368980019005214.

Wenzl T, Lachenmeier DW, Gökmen V. 2007. Analysis of heat-induced contaminants (acrylamide, chloropropanols and furan) in carbohydrate-rich food. Anal Bioanal Chem. 389(1):119–137. doi:10.1007/s00216-007-1459-9.

World Health Organization 2002. Health Implications of Acrylamide in Food. Geneva: WHO.

Wyka J, Tajner-Czopek A, Broniecka A, Piotrowska E, Bronkowska M, Biernat J. 2015. Estimation of dietary exposure to acrylamide of Polish teenagers from an urban environment. Food Chem Toxicol. 75:151–155. doi:10.1016/j.fct.2014.11.003.

Zając J, Bojar I, Helbin J, Kolarzyk E, Potocki A, Strzemecka J, Owoc A. 2013. Dietary acrylamide exposure in chosen population of South Poland. Ann Agric Environ Med. 20(2):351–355.

Zyzak DV, Sanders RA, Stojanovic M, Tallmadge DH, Eberhart BL, Ewald DK, Gruber DC, Morsch TR, Strothers MA, Rizzi GP, et al. 2003. Acrylamide formation mechanism in heated foods. J Agric Food Chem. 51(16):4782–4787. doi:10.1021/jf034180i.

Supplementary Material

Table S1: Attributed acrylamide levels (µg/100g of food), estimated based on the original distribution published in EFSA Scientific Opinion (14).

Food category	LC (%)	LOQ	Mean	P95
Potato fried products (except potato crisps and snacks)	14.3	2.4	35.4	97.1
French fries and potato fried, fresh or pre-cooked, sold as ready-to-eat	12.7	2.4	34.6	90.4
French fries and potato fried, fresh or pre-cooked, sold as fresh or pre- cooked, analysed as sold	40.5	2.4	60.8	188.8
French fries and potato fried, fresh or pre-cooked, sold as fresh or pre- cooked, prepared as consumed	15.3	2.4	27.9	65.6
French fries and potato fried, fresh or pre-cooked, sold as fresh or pre- cooked, preparation unspecified	15.6	2.4	42.8	146.8
Other potato fried products	2.1	2.4	61.9	154.9
Potato crisps and snacks	0.2	1.0	38.9	93.2
Potato crisps made from fresh potatoes	0.1	3.0	39.2	94.9
Potato crisps made from potato dough	0.5	3.0	33.8	75.0
Potato crisps unspecified	7.9	3.0	56.0	146.5
Potato snack other than potato crisp	4.3	3.0	28.3	28.3
Soft bread	48.8	2.0	7.0	15.6
Wheat soft bread	45.0	2.0	6.0	12.0
Other soft bread	40.2	2.0	8.5	24.0
Soft bread unspecified	64.2	2.0	8.7	14.1
Breakfast cereals	14.0	0.7	18.3	55.2
Maize, oat, spelt, barley and rice based products	24.8	0.7	12.8	40.3
Wheat and rye based products	5.3	0.7	17.8	41.0
Bran products and whole grains cereals	2.9	0.7	21.6	71.6
Breakfast cereals, unspecified	27.8	0.7	15.1	36.7
Biscuits, crackers, crisp bread and similar	18.0	3.0	31.8	104.8
Crackers	12.3	3.0	26.1	59.0
Crisp bread	20.8	3.0	21.0	48.6
Biscuits and wafers	21.1	3.0	25.0	81.0
Gingerbread	14.1	3.0	46.9	160.0
Coffee (dry)	3.5	4.0	54.0	105.4
Roasted coffee (dry)	6.9	4.0	26.6	54.3
Instant coffee (dry)	1.2	4.0	71.0	112.2
Coffee substitutes (dry)	6.8	8.0	149.9	450.0
Substitute coffee (dry), based on cereals	5.0	8.0	53.6	53.6
Substitute coffee (dry), based on chicory	0.0	8.0	294.2	294.2
Substitute coffee (dry), unspecified	16.1	8.0	49.3	49.3
Baby foods, other than cereal-based	66.8	1.0	5.1	7.2
Baby foods, not containing prunes	67.8	1.0	4.0	4.8
Infant formulae	97.0	1.0	10.0	10.0
Fruit purée	62.5	1.0	4.0	4.0
Juice	100.0	1.0	0.0	0.0
Ready-to-eat meal and dessert	64.3	1.0	3.6	5.1
Baby foods, containing prunes	0.0	1.0	10.1	10.1

Baby foods, unspecified regarding prunes content	78.3	1.0	11.5	11.5
Processed cereal-based baby foods	28.0	2.0	9.7	17.5
Biscuits and rusks_baby foods	28.1	2.0	14.7	28.7
Other processed cereal-based foods_baby foods	55.2	2.0	18.8	6.0
Cereals to be reconstituted_baby foods	54.7	2.0	26.3	8.6
Ready-to-eat meal cereal-based_baby foods	56.2	1.0	1.8	3.0
Unspecified processed cereal-based foods_baby foods	4.5	2.0	2.6	8.3
Other products based on potatoes, cereals and cocoa	32.7	3.0	13.7	37.0
Porridge	22.2	3.0	3.6	3.6
Cake and pastry	30.8	3.0	8.8	21.9
Savoury snacks other than potato-based (mostly maize-based)	13.3	3.0	19.4	69.0
Other products based on cereals	53.1	3.0	13.0	29.3
Grains for human consumption	56.2	3.0	8.9	15.2
Grains milling products	35.3	3.0	17.3	17.3
Pasta	88.9	3.0	0.0	0.0
Beer	100.0	3.0	0.0	0.0
Composite dishes containing cereals	32.0	3.0	17.9	17.9
Fine bakery wares for diabetics	0.0	3.0	13.9	13.9
Other	28.6	3.0	14.6	14.6
Other (non-fried) products based on potatoes	60.0	3.0	26.0	26.0
Potato bread	0.0	3.0	57.0	57.0
Other	64.9	3.0	18.8	18.8
Other products based on cocoa	11.4	3.0	11.6	11.6
Cocoa powder	7.7	3.0	19.3	19.3
Other products based on cocoa	12.9	3.0	8.3	8.3
Other products	45.8	3.0	59.2	151.0
Roasted nuts and seeds	55.0	3.0	18.2	18.2
Black olives in brine	0.0	3.0	45.4	45.4
Prunes and dates	27.8	3.0	12.0	12.0
Vegetable crisps	9.1	3.0	202.8	202.8
Paprika powder	56.7	3.0	84.3	84.3
Other	55.6	3.0	13.3	13.3

Abbreviations: LC – left-censored samples; P95 – 95th percentile; LOQ - limits of quantification

Table S2: Associations between food groups (g) and the usual daily exposure to acrylamide (µg/kg of body weight/day) among children and adolescents (n = 1959), weighted for the complex survey design. Linear regression models fit, after log-transforming and standardisation, were used to obtain RD for the standard deviation of the food group consumption and the respective 95% CI.

		Crude model	Model 1	Model 2
Food groups	**SD**	**RD (95% CI)**	**RD (95% CI)**	**RD (95% CI)**
Fresh and processed vegetables	78.9	0.95 (0.90, 1.02)	0.97 (0.92, 1.03)	0.95 (0.90, 1.01)
Nuts, oilseeds and processed products	6.18	**1.03 (1.00, 1.07)**	1.03 (0.99, 1.08)	**1.04 (1.01, 1.07)**
Fresh fruit and fruit jars	103	0.98 (0.92, 1.05)	1.02 (0.98, 1.07)	1.00 (0.95, 1.04)
Processed fruit	6.74	**0.97 (0.94, 1.00)**	**0.98 (0.95, 1.00)**	0.99 (0.96, 1.01)
Legumes	17.6	**0.95 (0.90, 1.00)**	**0.95 (0.90, 1.00)**	**0.95 (0.91, 0.99)**
Milk	234	**1.08 (1.01, 1.15)**	1.05 (0.99, 1.11)	1.00 (0.94, 1.05)
Human milk	166	0.99 (0.94, 1.05)	0.99 (0.93, 1.05)	0.98 (0.93, 1.04)
Infant formula	168	0.99 (0.94, 1.04)	0.99 (0.94, 1.04)	0.98 (0.93, 1.03)
Milk cream	5.19	0.99 (0.95, 1.04)	**0.96 (0.92, 1.00)**	**0.96 (0.92, 1.00)**
Yoghurt and other fermented milk	89.1	1.01 (0.95, 1.07)	1.01 (0.96, 1.07)	0.99 (0.95, 1.04)
Cheese	22.8	1.01 (0.95, 1.07)	1.01 (0.96, 1.07)	0.99 (0.95, 1.04)
Pasta	42.2	**0.93 (0.87, 0.99)**	**0.92 (0.88, 0.96)**	**0.96 (0.91, 1.00)**
Rice and other grains	75.5	**0.90 (0.85, 0.95)**	**0.90 (0.85, 0.94)**	**0.94 (0.90, 0.99)**
Potatoes and other starchy tubers	56.9	**1.05 (1.00, 1.11)**	1.00 (0.95, 1.06)	**1.05 (1.00, 1.10)**
Bread and rusks	58.2	0.99 (0.93, 1.06)	1.03 (0.97, 1.10)	**1.08 (1.02, 1.14)**
Flour, bread dough and pastry dough	15.3	1.01 (0.95, 1.09)	0.98 (0.93, 1.05)	1.03 (0.97, 1.08)
Infant cereals	84.8	1.03 (0.98, 1.08)	1.04 (0.98, 1.09)	1.04 (0.99, 1.09)
Breakfast cereals and cereal bars	22.6	**1.08 (1.02, 1.15)**	**1.09 (1.04, 1.15)**	**1.15 (1.10, 1.20)**
Meat and processed meat	77.7	**0.93 (0.87, 0.99)**	**0.94 (0.88, 1.00)**	0.99 (0.94, 1.05)
Fish, processed fish and seafood	35.1	0.99 (0.94, 1.05)	0.99 (0.94, 1.05)	0.99 (0.94, 1.04)
Eggs	19.0	1.01 (0.96, 1.08)	0.98 (0.93, 1.02)	0.99 (0.95, 1.03)
Vegetable oils	3.73	**1.09 (1.01, 1.19)**	**1.10 (1.04, 1.17)**	**1.07 (1.01, 1.13)**
Olive oil	5.15	**0.94 (0.89, 1.00)**	0.98 (0.91, 1.04)	1.01 (0.95, 1.07)
Butter	5.81	1.00 (0.96, 1.04)	1.00 (0.96, 1.05)	1.00 (0.96, 1.03)
Margarines and minarines	4.20	0.97 (0.92, 1.03)	1.00 (0.96, 1.04)	1.01 (0.97, 1.05)
Other fats	1.43	0.95 (0.90, 1.01)	0.97 (0.92, 1.03)	0.99 (0.94, 1.04)
Sweets	38.0	**1.08 (1.01, 1.15)**	**1.07 (1.01, 1.12)**	1.04 (0.99, 1.09)
Cakes	32.3	**1.11 (1.07, 1.16)**	**1.11 (1.07, 1.15)**	**1.12 (1.09, 1.16)**
Cookies and biscuits	21.3	**1.20 (1.13, 1.27)**	**1.22 (1.16, 1.28)**	**1.23 (1.18, 1.28)**
Water	445	**0.88 (0.82, 0.93)**	**0.86 (0.81, 0.91)**	**0.92 (0.87, 0.97)**
Tea and infusions	41.3	**0.92 (0.86, 0.98)**	**0.93 (0.90, 0.97)**	**0.95 (0.91, 0.99)**
Coffee	13.8	1.01 (0.96, 1.07)	1.00 (0.95, 1.06)	1.03 (0.98, 1.08)

Natural and 100% fruit juices	50.8	0.97 (0.92, 1.02)	0.96 (0.92, 1.01)	0.97 (0.93, 1.02)
Nectars	68.2	0.99 (0.91, 1.07)	0.97 (0.92, 1.03)	0.99 (0.95, 1.03)
Soft drinks	178	0.99 (0.93, 1.04)	**0.92 (0.87, 0.97)**	0.98 (0.94, 1.03)
Other non-alcoholic beverages	3.64	0.98 (0.94, 1.02)	**0.96 (0.95, 0.98)**	**0.96 (0.95, 0.98)**
Wine	6.93	0.96 (0.90, 1.03)	0.98 (0.94, 1.02)	0.98 (0.95, 1.01)
Liquors	3.22	0.97 (0.91, 1.03)	0.97 (0.91, 1.03)	0.98 (0.93, 1.03)
Beer	9.16	1.00 (0.98, 1.03)	**1.04 (1.00, 1.08)**	1.02 (0.99, 1.05)
Spirits	0.48	**0.96 (0.92, 0.99)**	**0.96 (0.93, 0.99)**	**0.97 (0.94, 1.00)**
Other alcoholic beverages	1.71	**0.96 (0.94, 0.97)**	**0.97 (0.95, 0.99)**	**0.98 (0.96, 0.99)**
Artificial sweeteners	0.08	**0.94 (0.93, 0.96)**	**0.96 (0.94, 0.97)**	**0.96 (0.95, 0.98)**
Snacks and chips	11.0	**1.13 (1.07, 1.20)**	**1.11 (1.05, 1.17)**	**1.08 (1.02, 1.15)**
Savoury pies and finger foods	21.7	**1.08 (1.03, 1.13)**	**1.04 (1.00, 1.08)**	**1.06 (1.03, 1.09)**
Meat substitutes	5.16	0.97 (0.92, 1.01)	**0.96 (0.93, 0.99)**	**0.97 (0.94, 1.00)**
Milk and milk products substitutes	35.3	1.01 (0.94, 1.08)	1.02 (0.97, 1.06)	0.99 (0.96, 1.03)
Table salt	1.27	0.95 (0.90, 1.01)	1.00 (0.94, 1.07)	0.95 (0.89, 1.01)
Yeasts and gelatines	0.58	1.08 (0.96, 1.21)	1.03 (0.98, 1.08)	1.03 (0.98, 1.09)
Aromas	0.28	0.97 (0.91, 1.03)	0.97 (0.92, 1.02)	0.97 (0.93, 1.01)
Herbs and spices	1.05	0.98 (0.91, 1.06)	1.00 (0.95, 1.06)	1.00 (0.95, 1.05)
Condiments	1.78	**0.93 (0.88, 0.99)**	**0.93 (0.87, 1.00)**	**0.95 (0.90, 1.00)**
Sauces and mayonnaise	3.70	1.04 (0.98, 1.09)	1.01 (0.97, 1.06)	1.01 (0.96, 1.05)
Soups and powdered soups	2.40	0.98 (0.92, 1.03)	0.97 (0.93, 1.01)	0.98 (0.94, 1.02)

Abbreviations: SD – standard deviation; RD - relative mean differences; CI – confidence interval. Crude model – without adjustment; Model 1 – adjusted for all food groups; Model 2 – adjusted for model 1 and for sex, age groups and parent's educational level.
Significant values are in **bold**.

Table S3: Associations between food groups (g) and the usual daily exposure to acrylamide (µg/kg of body weight/day) among adults (n = 3852), weighted for the complex survey design. Linear regression models fit, after log-transforming and standardisation, were used to obtain RD for the standard deviation of the food group consumption and the respective 95% CI.

		Crude model	Model 1	Model 2
Food groups	**SD**	**RD (95% CI)**	**RD (95% CI)**	**RD (95% CI)**
Fresh and processed vegetables	103	0.98 (0.94, 1.01)	0.97 (0.93, 1.01)	**0.97 (0.93, 1.00)**
Nuts, oilseeds and processed products	12.1	**1.13 (1.10, 1.16)**	**1.09 (1.06, 1.11)**	**1.07 (1.05, 1.09)**
Fresh fruit and fruit jars	157	**0.97 (0.93, 1.00)**	**0.97 (0.95, 1.00)**	**0.97 (0.95, 1.00)**
Processed fruit	6.08	1.01 (0.98, 1.04)	1.01 (0.98, 1.03)	1.01 (0.98, 1.03)
Legumes	37.5	1.00 (0.97, 1.04)	**0.97 (0.95, 1.00)**	0.98 (0.96, 1.01)
Milk	172	**1.06 (1.03, 1.10)**	**1.04 (1.01, 1.08)**	**1.04 (1.01, 1.08)**
Milk cream	6.27	**1.08 (1.05, 1.11)**	1.01 (0.98, 1.04)	1.00 (0.98, 1.03)
Yoghurt and other fermented milk	89.8	1.02 (0.98, 1.06)	**1.03 (1.01, 1.05)**	1.01 (0.98, 1.03)
Cheese	27.1	**1.10 (1.06, 1.13)**	1.02 (0.99, 1.04)	1.01 (0.99, 1.04)
Pasta	45.6	**1.04 (1.01, 1.07)**	1.01 (0.98, 1.03)	1.01 (0.99, 1.04)
Rice and other grains	82.1	1.00 (0.97, 1.04)	**0.97 (0.94, 1.00)**	0.98 (0.95, 1.01)
Potatoes and other starchy tubers	85.6	**1.11 (1.07, 1.15)**	**1.09 (1.05, 1.13)**	**1.11 (1.07, 1.15)**
Bread and rusks	84.0	**1.20 (1.16, 1.24)**	**1.16 (1.12, 1.19)**	**1.17 (1.14, 1.21)**
Flour, bread dough and pastry dough	18.6	**1.13 (1.09, 1.17)**	**1.05 (1.02, 1.08)**	**1.05 (1.02, 1.08)**
Infant cereals	14.9	**1.03 (1.00, 1.05)**	1.01 (0.99, 1.03)	1.01 (0.99, 1.02)
Breakfast cereals and cereal bars	18.9	**1.14 (1.11, 1.17)**	**1.13 (1.10, 1.16)**	**1.12 (1.09, 1.15)**
Meat and processed meat	102	**1.17 (1.13, 1.20)**	**1.05 (1.01, 1.08)**	**1.04 (1.01, 1.08)**
Fish, processed fish and seafood	62.7	0.99 (0.96, 1.03)	0.98 (0.95, 1.01)	0.98 (0.95, 1.01)
Eggs	27.5	**1.06 (1.02, 1.10)**	**1.03 (1.00, 1.06)**	1.02 (0.99, 1.06)
Vegetable oils	4.49	**1.15 (1.07, 1.22)**	**1.08 (1.04, 1.12)**	**1.08 (1.04, 1.12)**
Olive oil	8.24	**1.05 (1.02, 1.09)**	1.02 (0.98, 1.07)	1.03 (0.99, 1.07)
Butter	7.68	**1.13 (1.08, 1.18)**	1.02 (0.99, 1.06)	1.01 (0.98, 1.05)
Margarines and minarines	5.45	**1.05 (1.01, 1.08)**	1.00 (0.97, 1.03)	1.00 (0.97, 1.03)
Other fats	2.72	**1.07 (1.03, 1.11)**	1.01 (0.99, 1.04)	1.01 (0.98, 1.03)
Sweets	43.6	**1.13 (1.09, 1.16)**	**1.04 (1.01, 1.07)**	**1.03 (1.00, 1.05)**
Cakes	33.0	**1.20 (1.16, 1.23)**	**1.13 (1.11, 1.15)**	**1.13 (1.11, 1.15)**
Cookies and biscuits	19.6	**1.21 (1.17, 1.24)**	**1.20 (1.17, 1.23)**	**1.19 (1.17, 1.23)**
Water	632	1.02 (0.98, 1.05)	0.99 (0.97, 1.02)	0.99 (0.97, 1.01)
Tea and infusions	204	0.97 (0.94, 1.01)	0.99 (0.96, 1.02)	0.98 (0.95, 1.02)
Coffee	85.4	**1.14 (1.08, 1.20)**	**1.15 (1.10, 1.20)**	**1.15 (1.10, 1.20)**
Natural and 100% fruit juices	59.1	1.03 (0.97, 1.09)	1.00 (0.97, 1.04)	1.00 (0.97, 1.03)
Nectars	65.3	**1.07 (1.03, 1.10)**	**1.03 (1.00, 1.05)**	1.02 (0.99, 1.05)

Soft drinks	175	**1.11 (1.07, 1.15)**	1.01 (0.98, 1.04)	1.01 (0.98, 1.04)
Other non-alcoholic beverages	26.3	**1.05 (1.03, 1.07)**	**1.03 (1.01, 1.06)**	**1.04 (1.02, 1.07)**
Wine	169	1.02 (0.99, 1.05)	1.00 (0.97, 1.03)	**1.04 (1.01, 1.07)**
Liquors	10.6	**1.06 (1.02, 1.11)**	0.98 (0.94, 1.02)	0.99 (0.95, 1.02)
Beer	195	**1.23 (1.20, 1.27)**	**1.17 (1.14, 1.21)**	**1.18 (1.15, 1.21)**
Spirits	9.85	**1.05 (1.03, 1.08)**	1.00 (0.98, 1.03)	1.00 (0.98, 1.03)
Other alcoholic beverages	44.4	**1.04 (1.01, 1.07)**	**1.02 (1.00, 1.04)**	**1.02 (1.00, 1.03)**
Artificial sweeteners	0.24	1.00 (0.97, 1.03)	1.00 (0.98, 1.03)	1.00 (0.98, 1.03)
Snacks and chips	9.84	**1.14 (1.10, 1.18)**	**1.08 (1.06, 1.11)**	**1.07 (1.05, 1.10)**
Savoury pies and finger foods	26.5	**1.15 (1.12, 1.18)**	**1.12 (1.09, 1.15)**	**1.12 (1.09, 1.14)**
Meat substitutes	7.39	1.04 (0.99, 1.08)	**1.02 (1.00, 1.04)**	1.01 (0.99, 1.04)
Milk and milk products substitutes	53.8	**1.03 (1.00, 1.06)**	**1.03 (1.00, 1.06)**	**1.03 (1.00, 1.05)**
Table salt	1.78	**1.09 (1.06, 1.12)**	0.98 (0.94, 1.01)	0.98 (0.94, 1.01)
Yeasts and gelatines	0.33	**1.08 (1.05, 1.11)**	1.01 (0.98, 1.04)	1.01 (0.98, 1.04)
Aromas	1.19	**1.02 (1.01, 1.04)**	1.00 (0.98, 1.01)	0.99 (0.98, 1.01)
Herbs and spices	1.80	**1.07 (1.03, 1.11)**	1.01 (0.98, 1.05)	1.00 (0.97, 1.04)
Condiments	4.12	1.02 (0.99, 1.05)	1.00 (0.98, 1.03)	1.00 (0.98, 1.03)
Sauces and mayonnaise	5.20	**1.09 (1.05, 1.13)**	1.00 (0.98, 1.03)	1.00 (0.97, 1.02)
Soups and powdered soups	4.56	1.01 (0.99, 1.04)	1.00 (0.98, 1.02)	1.00 (0.98, 1.02)

Abbreviations: SD – standard deviation; RD - relative mean differences; CI – confidence interval. Crude model – without adjustment; Model 1 – adjusted for all food groups; Model 2 – adjusted for model 1 and for sex, age groups and educational level.
Significant values are in **bold**.

Paper II

Methodological approaches for the assessment of bisphenol A exposure

Sofia Almeida Costa, Milton Severo, Daniela Correia, Catarina Carvalho, Vânia Magalhães, Sofia Vilela, Sara Cunha, Susana Casal, Carla Lopes, Duarte Torres

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Methodological approaches for the assessment of bisphenol A exposure

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ABSTRACT

Bisphenol A (BPA) is an endocrine disruptor used in food contact materials, by the application of polycarbonate plastics and epoxy resins. The main objective of this study is to compare the estimate of daily BPA exposure at 13 years of age and in the adult Portuguese population, using different methodological approaches, and assess the associations between this exposure and sociodemographic characteristics.

Methodology: Cross-sectional data of 13-years follow-up from a population-based birth cohort Generation XXI (GXXI) (n = 2804) and from the National Food, Nutrition and Physical Activity Survey (IAN-AF 2015–2016) (n = 3845, ≥18 years old) was used. Dietary information was collected through three food diaries for adolescents and two non-consecutive 24-hour-recalls for adults.

To estimate the daily exposure to BPA, three methodological approaches were used. "Food groups attribution" merged the food consumption data with the concentration of BPA in food groups. "Regression tree model" and "random forest" combined food consumption information with urinary BPA, measured in a subsample of 24-hour urine (in adolescents n = 216, and in adults n = 82), both used to predict BPA exposure in the remaining sample. The fit-index of the methodologies was assessed through the root mean square error (RMSE), mean absolute error (MAE) and Spearman correlation coefficient (ρ). Associations between BPA exposure and sociodemographic variables were tested by linear regression models, adjusted for sex, age groups (in adults) and educational level. Tolerable Daily Intake (TDI) of 0.2 ng/kg body weight (bw), recently proposed by the European Food Safety Authority (EFSA), was used for the risk characterization of BPA exposure.

Results: The "random forest" was found as the best methodology to estimate the daily BPA exposure (adolescents: RMSE = 0.989, MAE = 0.727, ρ = 0.168; adults: RMSE = 0.193, MAE = 0.147, ρ = 0.250). The median dietary BPA exposure, calculated by "food groups attribution", was 79.1 and 46.1 ng/kg bw/day for adolescents and adults, respectively, while "random forest" estimated a BPA exposure of 26.7 and 38.0 ng/kg bw/day. 99.9% of the Portuguese population presented a daily exposure above TDI. Male adolescents, females and higher educated adults, were those more exposed to BPA.

Conclusions: The estimated daily BPA exposure strongly depends on the methodological approach. Food groups attribution may overestimate the exposure while the random forest appears to be a better methodological approach to estimate BPA exposure. Nevertheless, for all methods, the Portuguese population presented an unsafe BPA exposure by largely exceeding the safe levels proposed by EFSA.

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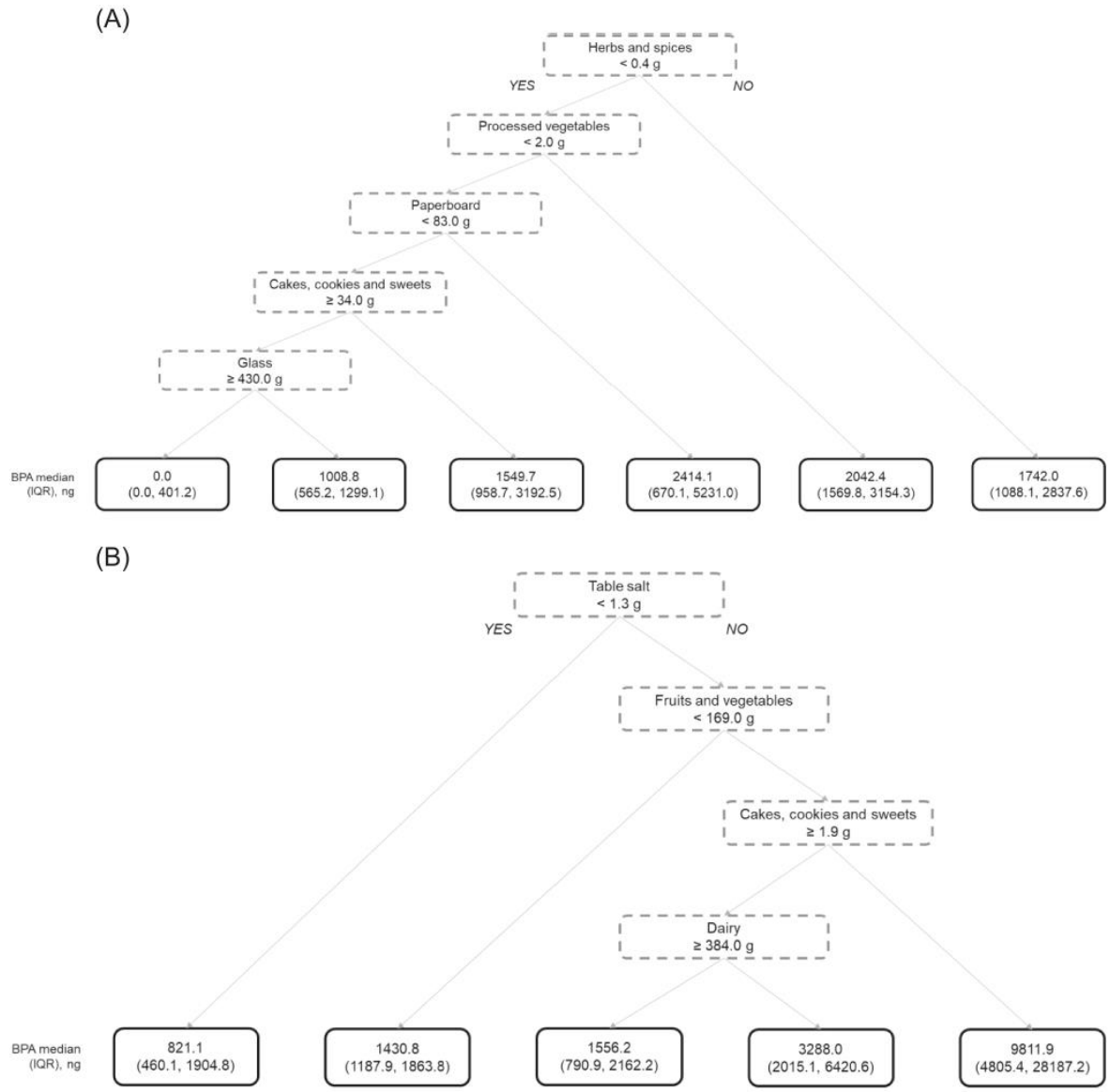

Fig. 1. Regression tree plot from the first imputation for adolescents' sample (n = 216) (A), and adults' sample (n = 82) (B). Variables of food groups and packaging materials (dashed line) used as predictors of absolute BPA, in nanograms (ng), measured in 24-hour urine samples (solid line).

1. Introduction

Bisphenol A (BPA) is a monomer widely used in the production of polycarbonate plastics and epoxy resins, as well as an additive for other polymeric materials. Due to their physical properties, polycarbonate plastics are applied to food contact materials, namely in reusable beverage bottles, infant feeding bottles, tableware, cookware, microwave ovenware, food containers and reservoirs for water dispensers (Vandenberg et al., 2007). Epoxy resins are frequently used in protective linings of food and beverage cans and as a coating for vats, storage tanks and supply systems (European Food Safety Authority, 2015). BPA has also been used to manufacture diverse non-food-related applications, such as toys, pacifiers, medical devices, thermal paper, printing inks, electronic products, and flame retardants (Kang et al., 2006; Olea et al., 1996; Pulgar et al., 2000).

Different studies concluded that individuals who consumed more food from packages containing BPA presented a higher concentration of this compound in their urine samples (Chang et al., 2019; Lakind & Naiman, 2011; Liu et al., 2018; Rudel et al., 2011). Depending on the storage conditions (temperature, pH and contact surface), the BPA can leach the components of the packaging materials and migrate into food and beverages (Cacho et al., 2012; Chang et al., 2019; Kang et al., 2006; Noureddine El Moussawi, Ouaini, et al., 2019; Noureddine El Moussawi, Cladière, Chébib, Ouaini, & Camel, 2019; Schecter et al., 2010; Sungur et al., 2014). Thus, diet is the most important source of BPA exposure across all population groups (European Food Safety Authority, 2015; Vandenberg et al., 2007; Von Goetz et al., 2010).

During the last years, a large amount of evidence has been published suggesting potential adverse health effects for humans due to BPA chronic exposure, even at low doses (Rochester, 2013; Vandenberg

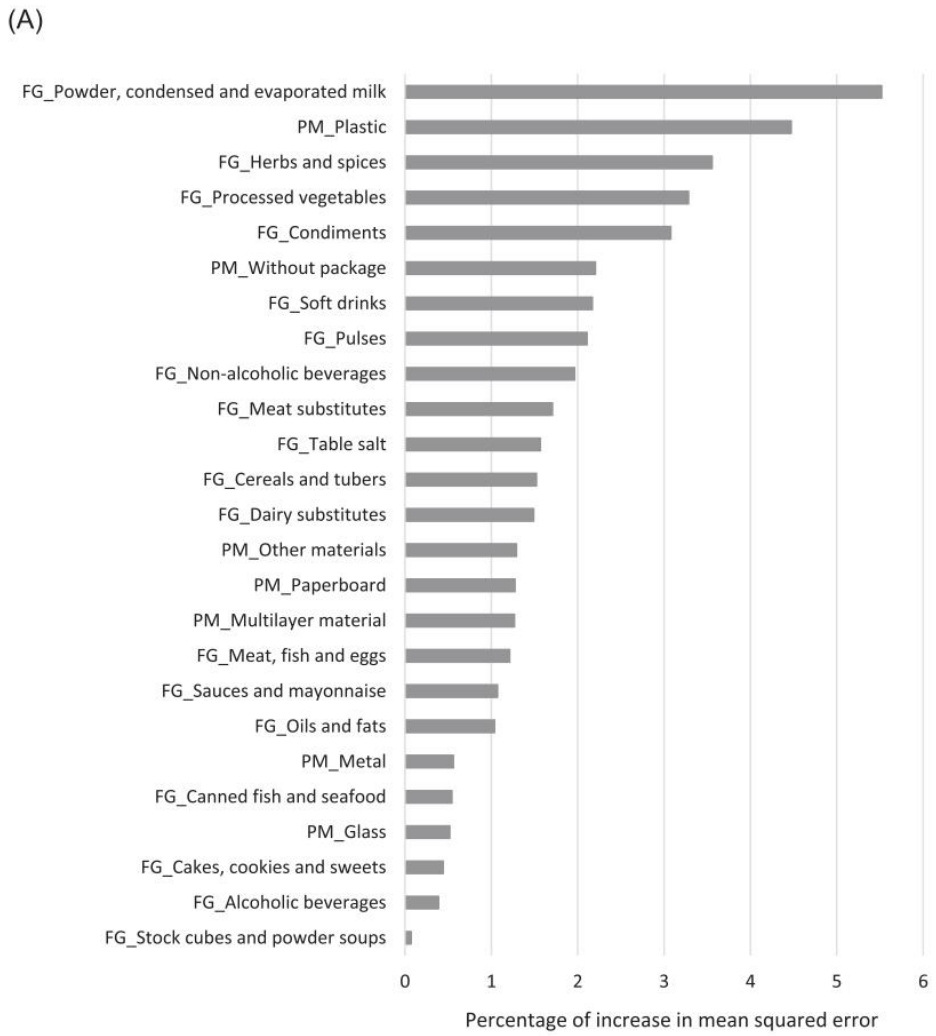

Fig. 2. Percentage of increase in mean squared error according to random forest variables importance for adolescents' sample (n = 216) (A), and for adults' sample (n = 82) (B). Plots from the first imputation.[11]

et al., 2007). This includes endocrine system deregulation, metabolic syndrome, obesity, diabetes mellitus, cardiotoxicity, hyperuricemia, immunological disabilities, reproductive complications and neuro-developmental/ neuroendocrine effects (Bao et al., 2020; Maffini et al., 2006; Rochester, 2013).

In that context, and based on the precautionary principle, the European Commission regulated the use of BPA in food contact materials (Regulation EU 10/2011 (European Commission, 2011b)), and the use of BPA in all plastic infant feeding bottles was restricted by the Regulation EU 321/2011 (European Commission, 2011a). In February 2018, the specific migration limit of BPA was updated to 0.05 mg/kg of food (Regulation EU 213/2018 (European Commission, 2018)). Moreover, in 2015, the European Food Safety Authority (EFSA) established a temporary Tolerable Daily Intake (t-TDI) of 4 μg/kg of body weight/day, pending on the outcomes of future long-term studies (European Food Safety Authority, 2015). But in 2023, EFSA has re-evaluated the risks to public health related to the presence of BPA in foodstuffs and recently proposed an updated TDI-value of 0.2 ng/kg of body weight (bw) (European Food Safety Authority, 2023; European Food Safety Authority Panel on Food Contact Materials Enzymes and Processing Aids, 2021).

Despite its hydrophobic characteristic (Hansch et al., 1995; Lide, 2005), BPA has no evidence of persisting in the environment or animal tissues (Cousins et al., 2002). After exposure, BPA is rapidly and extensively metabolized in the liver through conjugation reactions, being subsequently excreted in the urine, with a half-life of fewer than 6 h in the human body (Huang et al., 2017; Stahlhut et al., 2009; Thayer et al., 2015). BPA is completely eliminated via urine (Thayer et al., 2015), allowing the estimation of the daily exposure through daily urinary excretion (Vandenberg et al., 2007). Furthermore, different authors concluded that the level of BPA in the 24-h urine sample is similar to the level of dietary exposure to this contaminant due to the lower contribution of other exposure sources (Maffini et al., 2006; Meslin et al., 2022; Von Goetz et al., 2010).

Dietary exposure to food contaminants is usually assessed by indirect methods, such as food frequency questionnaires, 24-hour recalls and food diaries (de Fátima Poças & Hogg, 2007). Nevertheless, direct methods, namely measuring urinary biomarkers of exposure, can be used jointly with the traditional indirect approaches to better estimate

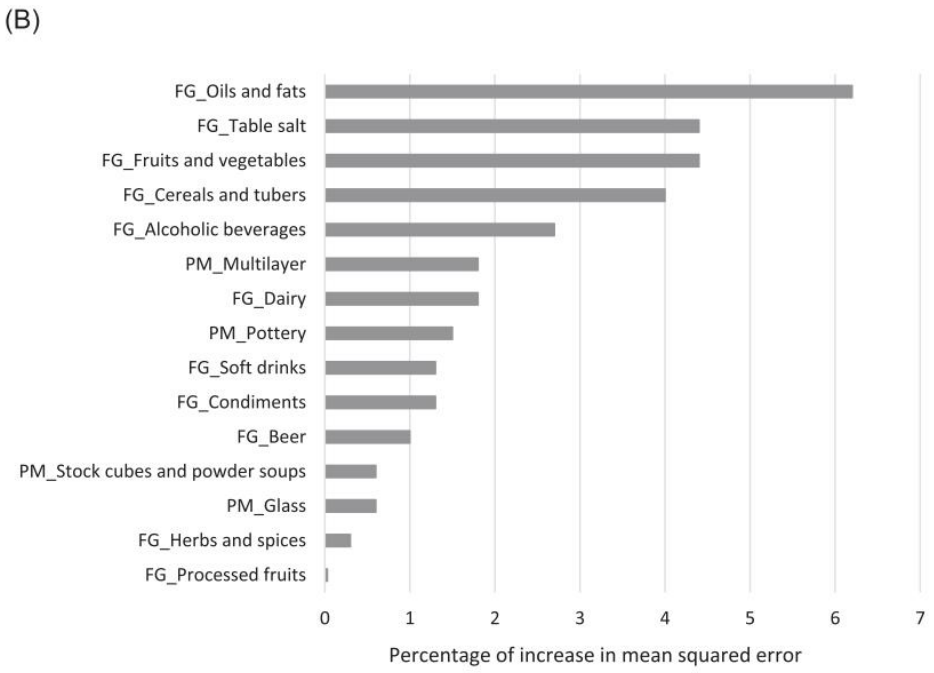

Fig. 2. (*continued*).

Table 1
Fit-index between bisphenol A measured in 24-hour urine samples, after box-cox transformation, and daily exposure to bisphenol A, using different methodological approaches for the exposure assessment.

	RMSE	MAE	ρ (95% CI)
Adolescents			
Full data			
Food groups attribution* *(n = 220)*	0.669	0.541	0.128 (-0.045, 0.294)
Regression tree** *(n = 216)*	0.909	0.684	0.482 (0.373, 0.578)
Random forest** *(n = 216)*	0.584	0.427	0.979 (0.972, 0.984)
Cross-validation 5 folds			
Regression tree** *(n = 216)*	1.044	0.790	0.093
Random forest** *(n = 216)*	0.989	0.727	0.168
Adults			
Full data			
Food groups attribution* *(n = 82)*	0.220	0.172	0.227 (-0.040, 0.464)
Regression tree** *(n = 82)*	0.152	0.118	0.632 (0.481, 0.746)
Random forest** *(n = 82)*	0.118	0.089	0.959 (0.938, 0.974)
Cross-validation 5 folds			
Regression tree** *(n = 82)*	0.205	0.154	0.250
Random forest** *(n = 82)*	0.193	0.147	0.250

Abbreviations: RMSE – root mean square error; MAE – mean absolute error; ρ – Spearman correlation coefficient; CI – confidence interval.
The random forest was the best methodology since it presented lower values for RMSE and MAE and a higher correlation coefficient compared to the regression tree and food groups attribution, for both adolescents and adults, in full data analysis and after the cross-validation.
* mean values of 5 imputations.
** values of the first imputation.

the exposure to food contaminants (Beckmann et al., 2020; Brouwer-Brolsma et al., 2017). In fact, limitations of indirect methods related to misreporting, participation bias, and identification of all sources and/or food contaminants occurrence data, can be overcome by direct methods that can provide objective estimates of xenobiotics exposure (Bingham, 2002; Brouwer-Brolsma et al., 2017; Gorecki et al., 2017).

Considering the recent EFSA scientific opinion and the new TDI value, there is a significant public health concern regarding dietary exposure to BPA for the entire population since the safety threshold was

[1] Abbreviations: FG – food group; PM – packaging material.

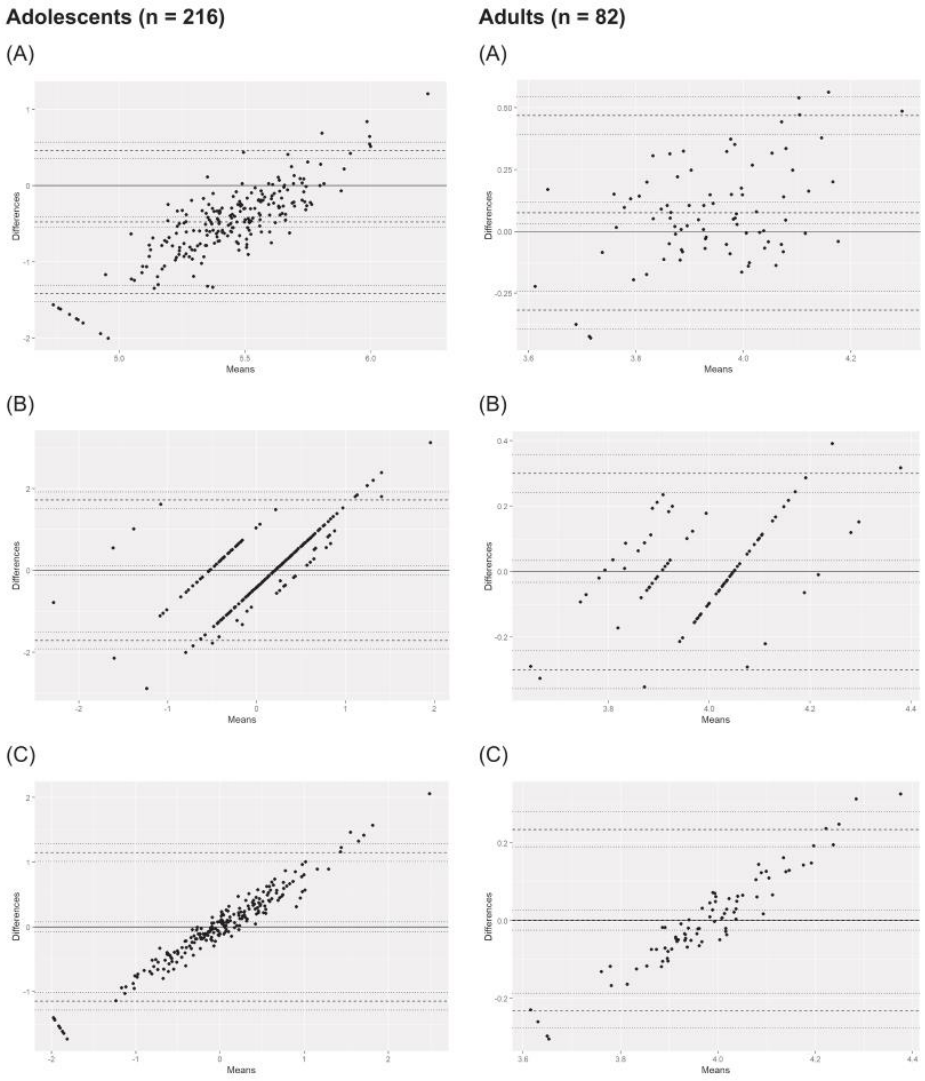

Fig. 3. Bland-Altman plots from the first imputation.[21]

largely exceeded (European Food Safety Authority, 2023; Meslin et al., 2022). However, this EFSA estimation could misrepresent the current dietary exposure (European Food Safety Authority, 2023), becoming essential to apply different methods to assess the dietary exposure to BPA further and characterize the related risk.

The present study aims to compare three different methodological approaches to estimate daily BPA exposure at 13 years of age and in the adult Portuguese population. Secondly, the best methodological approach will be used to assess the associations between BPA exposure and sociodemographic characteristics in both adolescents at 13 years of age and in the adult Portuguese population.

2. Methods

2.1. Study design and participants

This project included data from two population studies with available dietary information and urine samples: adolescents from a Portuguese population-based birth cohort and adults from a Portuguese national survey.

The adolescent sample was from Generation XXI (GXXI), an ongoing prospective population-based birth cohort, assembled between April 2005 and August 2006 at the five public units providing obstetrical and neonatal care in the metropolitan area of Porto, Portugal, as previously described (Larsen et al., 2013). In the 24 to 72 h after delivery, 91% of all invited mothers accepted to enrol in the cohort GXXI, resulting in 8,495 mothers and 8,647 children. Subsequently, all cohort participants were invited to participate in the 4-, 7-, 10- and 13-year follow-ups, with participation rates of 86%, 80%, 76% and 54%, respectively. It is important to highlight that the 13-year follow-up was interrupted in March 2020 due to issues related to the COVID-19 pandemic, reflected in the lower participation rate observed during this wave. This study used cross-sectional data from the 13-year follow-up which collected 24-hour urine (n = 240) and with complete information on food diaries (n = 2804).

Data from the National Food, Nutrition and Physical Activity Survey (IAN-AF 2015–2016) was used to assess the exposure to BPA in the adult Portuguese population. This survey included a representative sample of

Table 2
Validation of the different methodological approaches used to estimate the exposure to bisphenol A (ng/ day), by sex, educational level and body mass index categories. Differences were assessed through the Mann-Whitney test.

	Female	Male	*p-value*	≤12y of school[a]	>12y of school[a]	*p-value*	Underweight/ normal weight	Pre-obesity / obesity	*p-value*
Adolescents (n = 216)[b]									
Urine, median (IQR)	**1156 (1692)**	**1601 (2213)**	**0.002**	1481 (1901)	1456 (1794)	0.58	**1316 (1640)**	**1827 (2431)**	**0.005**
Food groups attribution, median (IQR)	**7060 (3095)**	**7770 (3222)**	**<0.001**	7439 (3127)	7255 (3382)	0.26	7392 (3222)	7390 (3236)	0.66
Regression tree, median (IQR)	1731 (948)	1731 (7)	0.60	1731 (478)	1731 (7)	0.87	1731 (7)	1731 (948)	0.13
Random forest, median (IQR)	**1311 (426)**	**1471 (523)**	**<0.001**	1359 (541)	1381 (425)	0.92	1385 (520)	1343 (569)	0.33
Adults (n = 82)									
Urine, median (IQR)	2074 (2316)	2818 (2962)	0.39	2445 (4512)	2909 (1563)	0.73	2462 (5152)	2881 (2031)	0.85
Food groups attribution, median (IQR)	**3043 (1709)**	**4739 (1699)**	**<0.001**	3889 (2260)	3999 (2695)	0.88	3766 (2244)	3909 (2449)	0.65
Regression tree, median (IQR)	4003 (2018)	4003 (2018)	0.21	4003 (2018)	4003 (2161)	0.31	4003 (2305)	4003 (2018)	0.71
Random forest, median (IQR)	2570 (1166)	3038 (1851)	0.10	2686 (1692)	2643 (720)	0.75	2585 (2305)	2794 (1208)	0.88

Abbreviations: IQR – interquartile range.
Significant values are in **bold**.
[a] Parent's educational level for adolescents.
[b] Values of the first imputation.

Table 3
The daily exposure of bisphenol A (ng/ kg of body weight/ day), estimated by applying different methodological approaches.

	Food groups attribution *median (IQR)*	Regression tree *median (IQR)*	Random forest *median (IQR)*
Adolescents (n = 2804)			
Total	79.1 (61.1, 102.2)	31.6 (22.0, 37.9)	26.7 (22.1, 31.8)
Sex			
Female	75.3 (58.1, 96.9)	31.7 (22.5, 37.4)	26.2 (22.0, 30.8)
Male	83.6 (64.5, 107.2)	31.5 (21.7, 38.4)	27.3 (22.4, 32.7)
Parents' educational level			
None, 1st and 2nd cycle	77.4 (60.2, 98.0)	30.5 (19.9, 37.0)	26.5 (20.7, 32.0)
3rd cycle and high school	78.8 (61.1, 101.9)	31.2 (20.3, 37.4)	26.6 (22.1, 31.5)
Higher education	81.0 (62.8, 103.6)	32.4 (24.7, 38.6)	27.1 (23.0, 32.0)
Adults (n = 3845)*			
Total	46.1 (33.1, 63.9)	41.7 (27.5, 58.9)	38.0 (32.1, 44.8)
Sex			
Female	41.4 (29.3, 55.1)	42.0 (27.1, 62.4)	39.3 (32.9, 47.0)
Male	52.3 (39.1, 72.5)	41.4 (28.2, 56.9)	37.0 (31.1, 42.2)
Age groups			
18–64 years	49.2 (36.0, 66.9)	41.3 (27.5, 57.8)	38.5 (32.5, 45.4)
65–84 years	36.4 (25.8, 49.7)	43.7 (29.3, 62.3)	36.0 (30.5, 42.5)
Educational level			
None, 1st and 2nd cycle	38.7 (27.7, 54.3)	42.1 (29.2, 60.4)	35.5 (30.1, 43.0)
3rd cycle and high school	49.6 (36.4, 67.2)	40.7 (26.8, 56.8)	38.5 (33.3, 44.9)
Higher education	50.0 (37.8, 65.0)	44.1 (27.6, 61.0)	39.3 (33.3, 46.8)

* Adults' results were weighted for the complex survey design.

the Portuguese population between 3 months and 84 years old, randomly selected by a multistage sampling methodology, as previously described (Lopes et al., 2017, 2018). Individual information of adult participants (≥18 years old) with two completed interviews (n = 3852) and with 24-h urine samples (n = 95) was used for the current analysis.

2.2. Anthropometrics measurements

Anthropometric measurements were performed under standard procedures, after 12 h of fasting, with subjects in light clothing and barefoot (Guerra et al., 2014). For both GXXI and IAN-AF 2015–2016 participants (Oliveira et al., 2018), the height was measured to the nearest centimetre using a wall stadiometer (SECA, Hamburg, Germany) and body weight to the nearest tenth of a kilogram using a digital scale (SECA, Columbia, USA). In the present study, 7 adult participants were excluded due to missing values of body weight measurement.

The body mass index (BMI) was calculated as weight over the squared height and adults were classified into the categories defined according to standards of the World Health Organization (WHO): underweight, normal weight, overweight and obesity. Adolescents' body mass index (BMI) was classified according to age and sex-specific BMI z-scores published by WHO (WHO Multicentre Growth Reference Study-Group., 2006).

2.3. Dietary assessment methods

Adolescents from GXXI were invited to complete a 3-day food diary before the face-to-face interview, helped by their parents/main caregiver and a set of oral and written instructions given by the research team. The participants described each food, drink and supplement consumed, including brands, packaging materials, food preparation method and consumption place. The food diaries were reviewed by trained researchers during the face-to-face interview and codified into the "eAT24" software, previously developed for the IAN-AF 2015–2016, described in detail bellowing in the text. The present study included participants that reported at least two completed days in the food diary.

[2] (A) Food groups attribution; (B) Regression tree; (C) Random forest.

Table 4
Associations between the daily exposure to bisphenol A (ng/ kg of body weight/ day), with socio-demographic variables in adolescents (n = 2804) and adults (n = 3845).

	Regression tree		Random forest	
	Crude model	Model 1	Crude model	Model 1
Adolescents	MD (95% CI)	MD (95% CI)	MD (95% CI)	MD (95% CI)
Sex				
Female	ref	ref	ref	ref
Male	0.74	0.72	**1.14**	**1.34**
	(-2.12, 3.60)	(-1.42, 2.84)	**(0.57, 1.70)**	**(0.62, 2.06)**
Parents' educational level				
None, 1st and 2nd cycle	ref	ref	ref	ref
3rd cycle and high school	−0.66	−0.70	0.18	0.11
	(-6.67, 5.35)	(-6.70, 5.30)	(-0.85, 1.21)	(-0.91, 1.13)
Higher education	1.10	1.08	0.73	0.70
	(-4.74, 6.94)	(-4.76, 6.91)	(-0.34, 1.81)	(-0.38, 1.77)
Adults*				
Sex				
Female	ref	ref	ref	ref
Male	0.97	0.97	**0.94**	**0.94**
	(0.93, 1.01)	(0.94, 1.01)	**(0.93, 0.95)**	**(0.93, 0.96)**
Age groups				
18–64 years	ref	ref	ref	ref
65–84 years	**1.07**	**1.08**	**0.97**	1.00
	(1.02, 1.12)	**(1.03, 1.14)**	**(0.95, 0.98)**	(0.98, 1.03)
Educational level				
None, 1st and 2nd cycle	ref	ref	ref	ref
3rd cycle and high school	**0.96**	0.98	**1.06**	**1.06**
	(0.92, 1.00)	(0.94, 1.03)	**(1.04, 1.08)**	**(1.04, 1.08)**
Higher education	**1.06**	**1.09**	**1.11**	**1.10**
	(1.01, 1.12)	**(1.04, 1.15)**	**(1.08, 1.13)**	**(1.08, 1.13)**

Abbreviations: BMI – body mass index; BPA – bisphenol A; MD – mean difference; CI – confidence interval; ref – reference category.
Crude model – without adjustment; Model 1 – adjusted for sex and parents' educational level in adolescents and adjusted for sex, age groups and educational level in adults.
Significant values are in **bold**.
* Adults' results were weighted for the complex survey design.

For the IAN-AF 2015–2016 adult participants, dietary information was collected in two face-to-face interviews, between October 2015 and September 2016, by trained interviewers in two non-consecutive 24-hour dietary recalls, with an interval of 8 to 15 days, following the EU Menu methodology proposed by EFSA (European Food Safety Authority, 2014). The collection and transformation of food data into nutrients resulted from using the validated "eAT24" software (Goios et al., 2020; Lopes et al., 2018), which integrates the Portuguese Food Composition Table and international data for missing items. All food, beverages and dietary supplements consumed by the participants in 24 h were reported, quantified and described as eaten according to the eating occasion. A complete description of all the food items reported was obtained by adopting the FoodEx2 classification system with their different facets and corresponding descriptors (European Food Safety Authority, 2011). This system allowed an extensive collection of information, namely the food packaging materials (pottery, wood, cork, glass, paper, paperboard, vegetal paper, film, plastic, textiles, multilayer materials, metal, aluminium, edible materials and other materials). Finally, foods, beverages and recipes were quantified through different methods available: weight (grams), volume (millilitres), food picture book (186 food photo series for foods/ recipes (6 portions each) and 11 photo series for household measures) (Torres et al., 2017), standard units, household measures and default portions.

2.4. Other variables

In GXXI, information regarding sociodemographic characteristics, namely sex and parents' educational level, was collected by trained interviewers at baseline and updated in each follow-up evaluation.

In the IAN-AF 2015–2016, demographic and socio-economic information, such as data on sex, age and educational level ("none, 1st and 2nd cycle", "3rd cycle and high school" and "higher education") was also collected.

2.5. Urine collection and chemical analysis

A convenience sample of 240 participants from the GXXI birth cohort was invited to collect urine for 24 h, as close as possible to the interview. Participants were selected based on the availability of complete food diary records throughout all follow-up waves, which occurred at 4, 7, 10, and 13 years. All procedures were explained, orally and by writing, to adolescents and respective parents/main caregivers, and the necessary material was made available, namely a BPA-free container.

As described previously, a sub-sample of 95 individuals from the IAN-AF 2015–2016 sampling frame was invited to participate in a validation study (Goios et al., 2020). These participants were instructed to bring a 24-hour urine sample at the second interview, corresponding to the second 24-hour dietary recall. Exclusion criteria were taking diuretics; having diabetes, kidney disease, haemophilia, or any condition requiring supplemental oxygen; donating blood or plasma during or less than 4 weeks before the study; being pregnant or lactating women; being under a dietary therapy; or having a urinary tract infection (Goios et al., 2020).

For all samples, urinary creatinine was measured by the Jaffe method (Beckman Coulter). Total BPA concentration levels were measured in urine using a dispersive liquid–liquid microextraction followed by gas chromatography coupled to mass spectrometry, as previously described (Cunha & Fernandes, 2010). The analytical method's detection and quantification limits were 0.03 ng/mL and 0.1 ng/mL, respectively (Cunha & Fernandes, 2010).

From the 240 samples from GXXI, 20 were not considered in the statistical analysis due to incomplete information regarding dietary consumption and additionally 4 for missing in the urinary output volume, ending with a sample of 216.

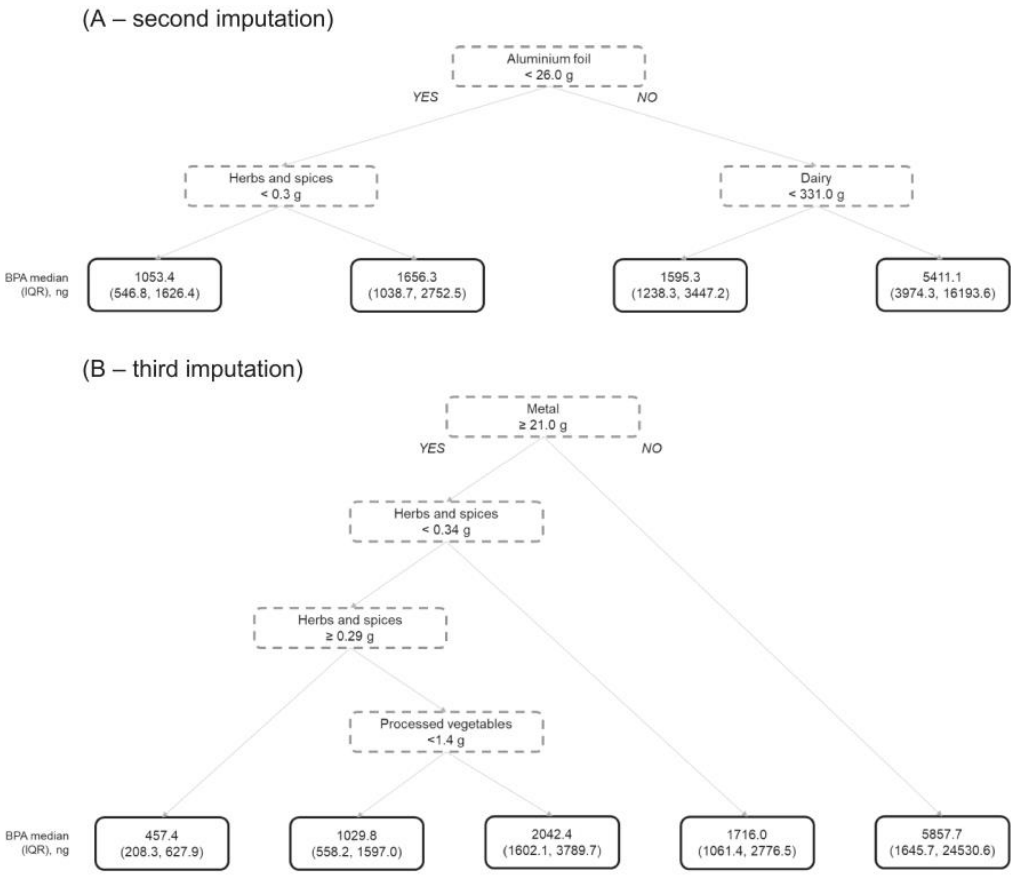

Fig. A1. Regression tree plot, from the second to the fifth imputation (A - D), for adolescents' sample (n = 216). Variables of food groups and packaging materials (dashed line) used as predictors of absolute BPA, in nanograms (ng), measured in 24-hour urine samples (solid line).

From the 95 urinary samples from IAN-AF 2015–2016, 9 were excluded due to their incomplete collection determined by the creatinine coefficient, i.e. creatinine excretion (mg/d) by body weight (kg) (if out of the parameters: 14.4 – 33.6 in males and 10.8 – 25.2 in females) (World Health Organization, 1984), and by the total volume of 24-hour urine collected (if less than 500 ml) (Wang et al., 2013). Moreover, 4 samples were excluded by the insufficient sampling volume available for laboratory analysis, leading to a final sample of 82.

2.6. Exposure assessment to BPA

To estimate the daily exposure to BPA, three methodological approaches were used. The first method, "food groups attribution", estimated the daily dietary exposure to BPA by merging the food consumption data with the concentration data of BPA in food groups, published by EFSA in 2015 (European Food Safety Authority, 2015). The EFSA scientific opinion considered BPA occurrence reported in the scientific literature, only considering studies of samples collected in Europe, and obtained through a specific EFSA calls for data. Occurrence data from foodstuffs collected in Portugal were considered, namely for canned products of "Fish and other seafood", "Non-alcoholic beverages", "Alcoholic beverages", "Food for infants and small children" and "Products for special nutritional use" food groups.

For each food group, according to the upper bound scenario, an average value of BPA concentration reported by EFSA was used to estimate the BPA exposure by adolescents from GXXI and adults from the Portuguese population. For this, a FoodEx2 code (base term and descriptor of packaging material facet, namely, canned or non-canned) was attributed to each food group reported in EFSA's publication (European Food Safety Authority, 2015). These codes were used to merge the occurrence data with the food consumption data, also codified with a base term and packaging material descriptor, according to the FoodEx2 classification system. In the end, the sum of exposure for each day by the individual was calculated, and then the daily average for the specific survey period was derived.

Additionally, two methods were developed combining the dietary information with urinary BPA: regression tree model and random forest, both used to predict the daily exposure to BPA in the remaining sample.

2.7. Risk characterization

Since BPA is rapidly metabolized, and there is a residual contribution of other exposure sources, it was assumed that the estimates gathered from regression tree model and random forest represent approximately daily dietary exposure to BPA. Thus, for the risk characterization of BPA exposure, the proportion of individuals above the TDI was calculated, for the three methodological approaches, using the recently proposed TDI value by the EFSA, 0.2 ng/kg bw (European Food Safety Authority, 2023).

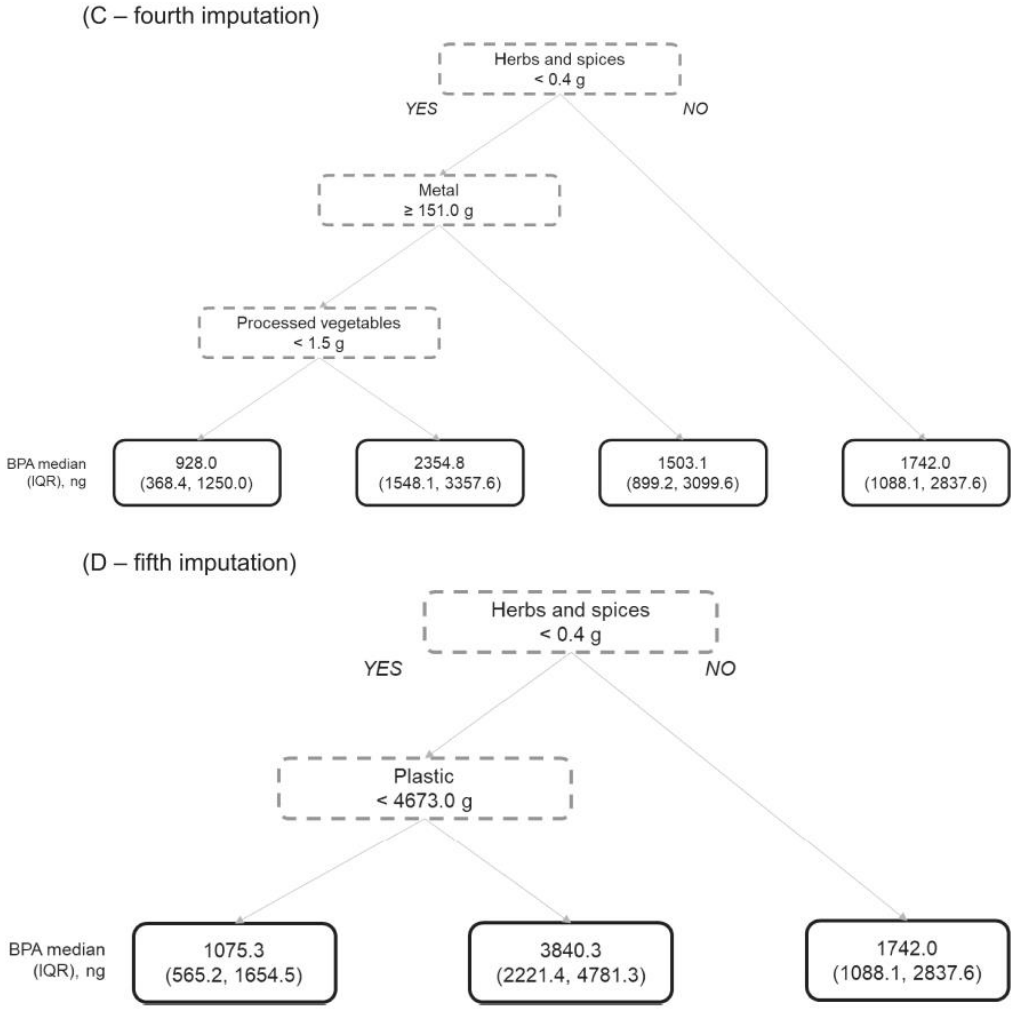

Fig. A1. (*continued*).

2.8. Ethics approval and consent to participate

The protocol of the GXXI birth cohort was approved by the Ethics Committee of São João University Hospital and registered with the Portuguese Individual Data Protection Authority. For IAN-AF 2015–2016, ethical approval was obtained from the National Commission for Data Protection, the Ethical Committee of the Institute of Public Health of the University of Porto and the Ethical Commissions of the Regional Administrations of Health. For GXXI participants, written informed consent was obtained from parents, or legal caregivers, and oral assent from adolescents. Written informed consent was obtained for adult participants from IAN-AF 2015–2016. Both studies complied with the national legislation and the Ethical Principles for Medical Research expressed in the Declaration of Helsinki. All documents with identification data were treated separately and stored in a different dataset. The databases with individual information are anonymized, allowing the non-identification of the participants. The Data Protection Officer at the Institute of Public Health of the University of Porto ensured data protection.

2.9. Statistical analysis

For GXXI and IAN-AF 2015–2016, to handle missing information on packaging material, a decision tree was used to generate a multiple imputation model (5 imputations), and combined according to Rubin's rule, as described elsewhere (Costa et al., 2021). The developed decision tree combined data from different variables such as participants' sex, age groups (in IAN-AF 2015–2016), educational level (or parents' educational level for GXXI), BMI categories, day of the dietary collection, eating place, eating occasion, food groups and packaging materials reported.

The multiple imputations did not significantly change the results for adults, so only the results of the first imputation are presented. However, slight differences were found for adolescents between the five imputations. Thus, the first imputation is presented in the manuscript, and the results of the second to the fifth imputation are in the supplementary material.

Total BPA excretion (ng/day) was calculated by multiplying the urinary BPA concentration (ng/L) and the urine volume (L/day), and compared with the BPA dietary exposure. A Box-Cox transformation was performed to approximate the distribution of the total BPA measured in urine to the normal distribution curve, applying a calculated exponent of

Fig. A2. Percentage of increase in mean squared error according to random forest variables importance. Plots from the second to the fifth imputation (A - D), for adolescents' sample (n = 216).[1]

−0.2 for adults and −0.1 for adolescents.

The fit-index between urinary BPA and the daily exposure, estimated using the three methodological approaches, was assessed through the root mean square error (RMSE), mean absolute error (MAE) and Spearman correlation coefficient (ρ). In addition, to handle the possible overfitting of the models, a repeated 2-times 5-fold cross-validation was performed.

Mann-Whitney test was used to test the median differences between urinary BPA and the three estimated daily exposure to BPA. This analysis was stratified by sex, educational levels and BMI categories. The variable educational level was dichotomized: "≤ 12 years of school" and "> 12 years of school". Finally, underweight and normal weight categories were grouped, as well as pre-obesity and obesity.

Bland-Altman plots were used to illustrate the difference between the three methods against the BPA measured in urine samples.

Associations between BPA exposure and sociodemographic characteristics were tested in 13 years old adolescents using linear regression models without transformation due to the approximated normal distribution, presented by mean differences (MD) and 95% confidence interval (CI). For adults, the differences were assessed by relative mean differences (RD) and the respective 95% CI, through linear regression models, after log-transforming the outcome due to its heavy-tailed distribution. Two separate models were applied: a crude model; and a model adjusted for sex, age groups (adults) and educational level (or

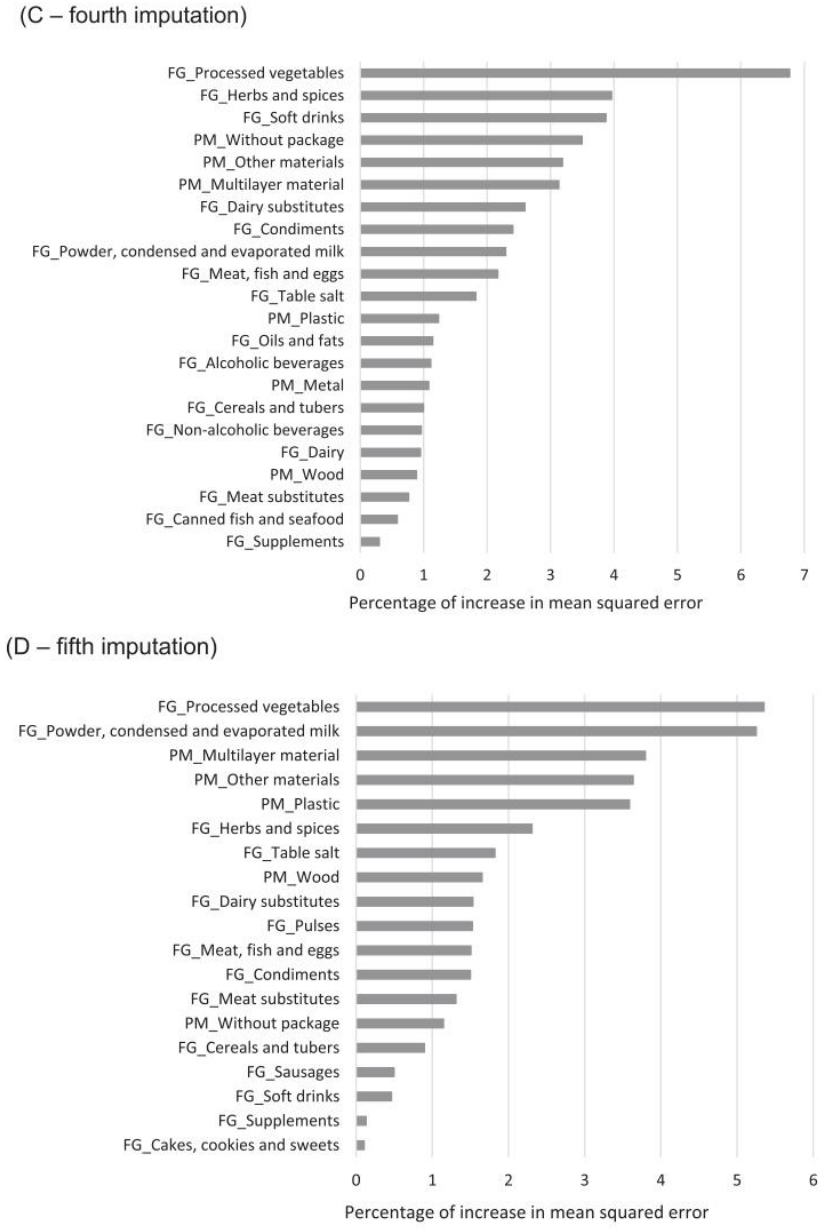

Fig. A2. (*continued*).

parent's educational level for adolescent sample) (model 1).

Analyses were performed using the R software version 4.2.1 for Windows, with a significance level of 5%.

3. Results

3.1. Development of regression tree and random forest

In this study, three methodological approaches were used to estimate the daily exposure to BPA in adolescents and adults: food groups attribution, regression tree and random forest.

Fig. 1 presents the regression tree plot of the first imputation, both for adolescents and adult samples. To estimate BPA exposure, the model used as predictors the food groups and the packaging material reported. Depending on the consumption in each decision node of the tree, participants were categorized into one terminal node of the possible branches, i.e. final output. For example, an adolescent was categorized in the lower BPA exposure group (131.1 ng/day) if consumed less than 0.4 g of herbs and spices, less than 2.0 g of processed vegetables, less than 83.0 g of food items packaged in paperboard, more or equal to 34.0 g of cakes, cookies and sweets and more or equal to 430.0 g of food items packaged in glass. But if the adolescent consumed more or equal to 0.4 g of herbs and spices the participant was categorized in the higher BPA exposure group (1732.7 ng/day) (see Fig. A1 for second to fifth imputation results, in supplementary material). The same interpretation is applied to adults; if they consumed less than 1.3 g of table salt, they were categorized in the lower BPA exposure group (821.1 ng/day). If they consumed more or equal to 1.3 g of table salt, more or equal to 169.0 g of

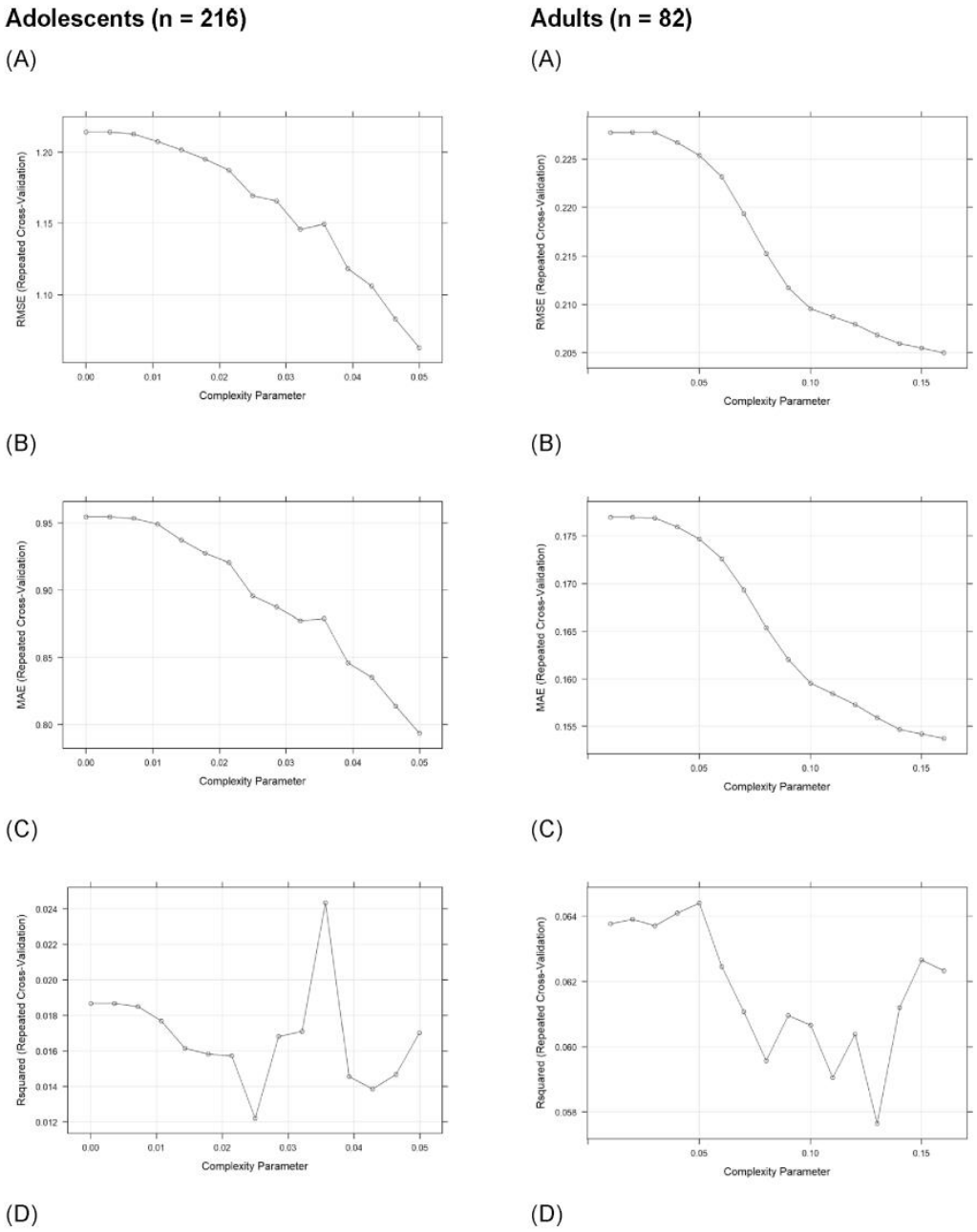

Fig. A3. Plots of fit-index parameters from the first imputation, after cross-validation (5 folds, repeated 2 times for adolescents and 50 times for adults), using different methodological approaches.[31]

fruits and vegetables and less than 1.9 g of cakes, cookies and sweets, they were categorized in the higher BPA exposure group (9811.9 ng/day).

Regarding the random forest, this methodology combines the various estimates of BPA exposure from multiple regression trees to reach a single result. In this study, 1000 uncorrelated regression trees were generated to create the random forest, using as predictors food groups and the packaging material reported. Fig. 2 shows the plot of the random forest variables' importance, in adolescents and adults, represented by the percentage of increase in the mean square error (%IncMSE) introduced in the model by changing those specific variables (see Fig. A2 for second to fifth imputation results, in supplementary material). For the first imputation, in adolescents, the consumption of powder, condensed and evaporated milk was the variable with higher importance for the estimation of BPA exposure (5.5 %IncMSE), followed by the consumption of food items packaged in plastic (4.5 %IncMSE) (Fig. 2). In adults, the most important variable was the consumption of oils and fats (6.2 % IncMSE), followed by table salt intake and the consumption of fruit and

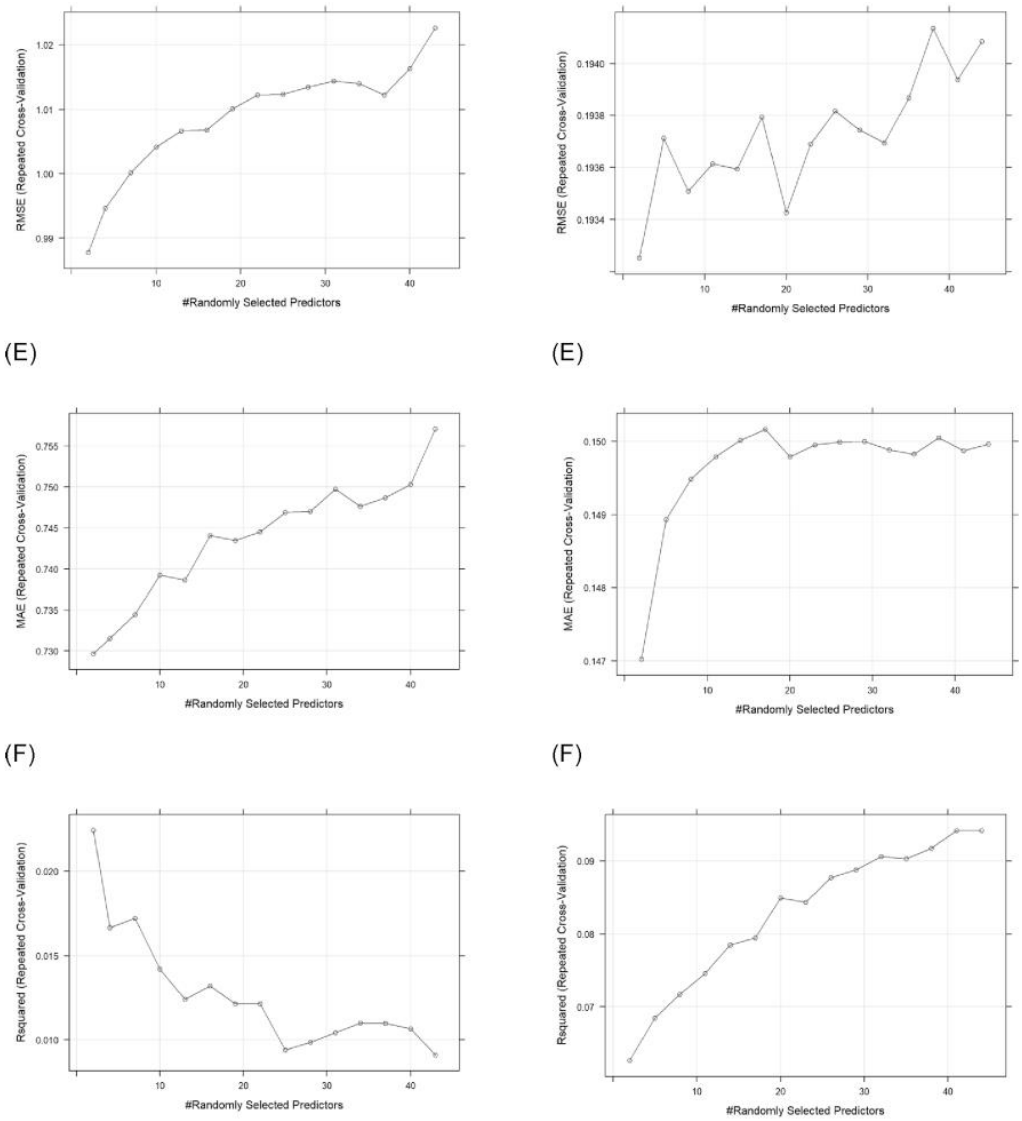

Fig. A3. (*continued*).

vegetables (4.4 %IncMSE, in both) (Fig. 2).

3.2. Fit of the exposure assessment methods

Table 1 presents the results for the fit-index between BPA measured in 24-hour urine samples, after box-cox transformation, and daily exposure to BPA, estimated by applying the three different methodological approaches. The random forest was the best methodology to estimate the daily exposure to BPA in our sample since it presented lower values for RMSE and MAE and a higher correlation coefficient compared to the regression tree and food groups attribution, for both adolescents and adults, in full data analysis and after the cross-validation (adolescents: RMSE = 0.989, MAE = 0.727, ρ = 0.168; adults: RMSE = 0.193, MAE = 0.147, ρ = 0.250). The plots of these parameters are presented in the supplementary material (figures A3 and A4). The Bland-Altman plot of the first imputation, for the three methodological approaches and full data analysis, in adolescents and adults, are presented in Fig. 3 (figure A5: second to the fifth imputation). Both for adolescents and adults, the food groups attribution plot shows a systematic bias, overestimating the exposure, and a large scatter of the differences, indicating that this approach was inaccurate for estimating BPA daily exposure (Fig. 3 - A). For the random forest (Fig. 3 – C), no systematic bias between exposure estimation and urinary excretion was observed. Nevertheless, the plot indicated possible overfitting using this model, confirming the need to perform cross-validation.

These results were corroborated by comparing BPA measured in urine samples with the value estimated by the different methodological approaches (Table 2). For adolescents and adults, the random forest was the methodology that better predict the daily exposure to BPA, presenting the closest values to the original BPA measured in urine and the

[3] (A) root mean squared error for regression tree; (B) mean absolute error for regression tree; (C) Spearman correlation coefficient for regression tree; (D) root mean squared error for random forest; (E) mean absolute error for random forest; (F) Spearman correlation coefficient for random forest

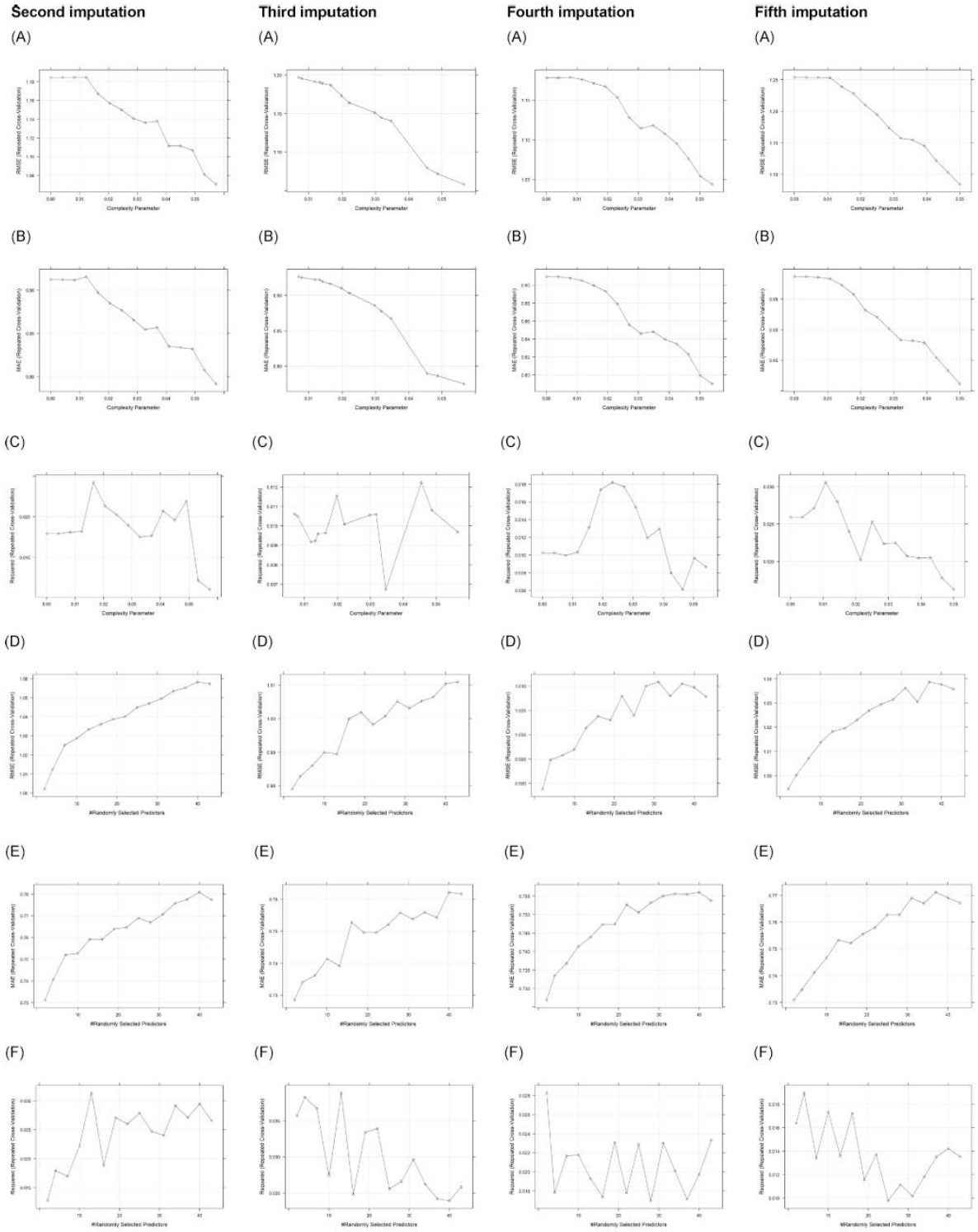

Fig. A4. Plots of fit-index parameters from the second to the fifth imputation, for adolescents' sample (n = 216), after cross-validation (5 folds, repeated 2 times), using different methodological approaches.[3]

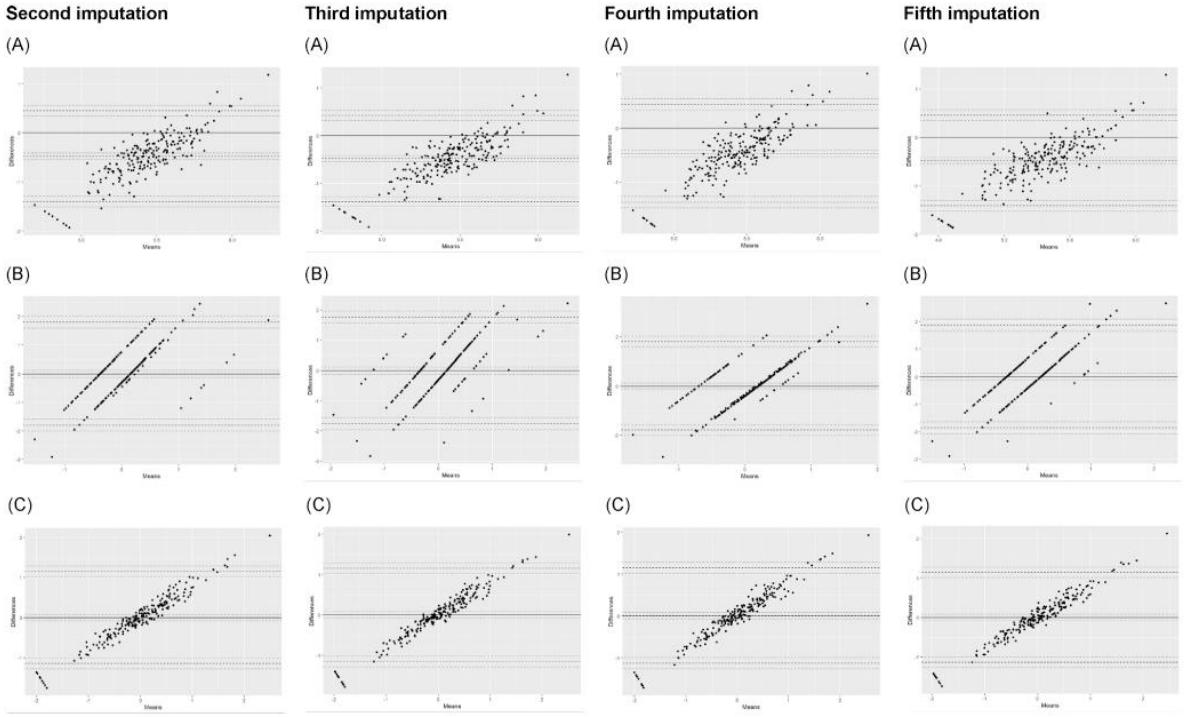

Fig. A5. Bland-Altman plots from the second to the fifth imputation, for adolescents' sample (n = 216).[2]

same tendency when the sample was stratified by sex, educational level and BMI categories. Moreover, food groups attribution was the methodology with the worst BPA daily exposure estimation, with the median values of BPA being 5–6 times higher than those measured in urine for adolescents and 1.5–2 times for adults.

3.3. BPA daily exposure assessment

Table 2 also shows that, in adolescents, males compared to females presented a significantly higher BPA value calculated from urinary samples (female = 1156 ng/day, male = 1601 ng/day, *p-value* = 0.003; random forest: female = 1311 ng/day, male = 1471 ng/day, *p-value* = less than0.001). Pre-obese and obese adolescents presented a significantly higher median exposure to BPA in urine samples, not detected in the remaining methodological approaches (female = 1316 ng/day, male = 1785 ng/day, *p-value* = 0.007).

In adults, according to food groups attribution, males consumed a significantly higher amount of BPA compared to females (female = 3043 ng/day, male = 4739 ng/day, p-value = less than0.001), not validated by urinary BPA.

Table 3 shows the exposure assessment of BPA, in ng/kg bw/day, estimated for the remaining adolescent sample and the adult population, according to "food groups attribution", regression tree and random forest. As expected by results from Table 2, compared to random forest estimates, food groups attribution overestimated the daily exposure to BPA in adolescents at 13 years of age and in adults from the Portuguese population. Using the "food groups attribution" methodological approach, the median daily dietary exposure to BPA in adolescents was 79.1 ng/kg bw/day, while 46.1 ng/kg bw/day in adults. The median value of BPA daily exposure in adolescents was 31.6 and 26.7 ng/kg bw/day for the regression tree and random forest, respectively. In the random forest approach, males were more exposed to BPA than females ($median_{female}$ = 26.2 ng/kg bw/day, $median_{male}$ = 27.3 ng/kg bw/day). In both methodologies, BPA daily exposure was higher in adolescents whose parents presented a higher educational level.

The median value of BPA daily exposure in adults was 41.7 and 38.0 ng/kg bw/day for the regression tree and random forest, respectively (Table 3). Males presented a lower exposure in the two approaches (regression tree: $median_{female}$ = 42.0 ng/kg bw/day, $median_{male}$ = 41.4 ng/kg bw/day; random forest: $median_{female}$ = 39.3 ng/kg bw/day, $median_{male}$ = 37.0 ng/kg bw/day), because of their higher body weight compared to females. Regarding age and educational level, the results differ according to the methodology. For random forest, the median of exposure to BPA decreased with age and increased in the most educated participants.

3.4. BPA risk characterization

Regarding risk characterization, in all estimates for all groups, independently of the methodological approach, 99.9% of the participants were above the TDI of 0.2 ng/kg bw, which may indicate a public health concern for these adolescents and the adults of the Portuguese population.

3.5. BPA exposure associated factors

Finally, Table 4 describes the associations between daily exposure to BPA and socio-demographic characteristics among adolescents and adults, respectively. With the random forest methodology, in adolescents, a statistically significant association between BPA exposure and sex was found. After adjustment for sex and parents' educational level (model 1), adolescent males were 0.34 ng/kg bw/day more exposed to BPA compared to females (MD = 1.34 [95%CI: 0.62, 2.06]). For adults, according to the random forest, in the adjusted model, it was observed an association between BPA exposure and sex and educational level. Males were 6% less exposed to BPA than females (RD = 0.94 [95%CI: 0.93, 0.96]). Moreover, BPA exposure was associated with higher educational levels (model 1), where individuals with 3rd cycle and high

school were 6% more exposed. Those with higher education were 10% more exposed to BPA, compared with those with lower education (RD = 1.06 [95%CI: 1.04, 1.08]; RD = 1.10 [95%CI: 1.08, 1.13], respectively).

4. Discussion

Usually, the research on human exposure to BPA focuses on food consumption estimates (with or without the combination of environmental sources) or urinary estimates rather than combining the information from direct and indirect methods. Moreover, most of the studies use urinary spot samples to assess the exposure to BPA, despite the sizeable intra-individual variability and the inconstant concentration of BPA in urine throughout the day, given the short half-life of this contaminant (Koch et al., 2014; LaKind & Naiman, 2008; Pollack et al., 2016). To the best of our knowledge, this was the first study comparing the estimates of BPA exposure using data from food consumption with 24-hour urinary excretion. The 24-hour urinary excretion of BPA is generally accepted as reflecting daily dietary exposure (15,30). Interestingly, in the regression models, only variables related to food consumption were selected.

The comparison with other studies is difficult considering the differences between the sample size, the sample representativeness, the protocol design, and the exposure assessment methodology. Regarding "food groups attribution", the median daily dietary exposure to BPA in the adolescent sample was 79.1 ng/kg bw/day, higher than the daily BPA exposure (expressed in a range for the median) estimated in 2014 and 2017 for the French adolescent population, from the Second National Individual Dietary Consumption Survey (Bemrah et al., 2014; Gorecki et al., 2017), which were, respectively, 42–46 and 39–41 ng/kg bw/day. The estimates from the present study are also 2–3 times above the daily dietary BPA exposure assessed in a non-representative sample of Korean adolescents (Park et al., 2016) and in a representative sample of Taiwanese adolescents (Chang et al., 2019). Nevertheless, in EFSA Opinion from 2015 (European Food Safety Authority, 2015), the daily average dietary exposure estimated for European adolescents was 159 ng/kg bw/day, almost two times above the BPA dietary exposure calculated in this paper. Compared to the Chinese Total Diet Study (Yao et al., 2020), the presented exposure was several times lower than the mean dietary BPA exposure calculated for this population (269.8 ng/kg bw/day and 321.1 ng/kg bw/day for females and males, respectively). However, the culture and the food patterns of Korea, Taiwan and China could be highly different from those observed in Western countries like Portugal, as previously discussed (Gerofke et al., 2023; Sakhi et al., 2014). Thus, these differences can be expected.

According to the food groups attribution methodology, the median daily dietary exposure to BPA for adults in the Portuguese population was 46.1 ng/kg bw/day. This result was in line with previous papers, namely compared to French Second National Individual Dietary Consumption Survey published in 2014 (range for the median: 29–31 ng/kg bw/day) and in 2017 (range for the median: 33–35 ng/kg bw/day) (Bemrah et al., 2014; Gorecki et al., 2017), and with Korean, Nigerian and Taiwanese studies (Adeyi & Babalola, 2019; Chang et al., 2019; Park et al., 2016), despite the differences observed in food habits between Portugal and the last countries. The Portuguese estimation was slightly above the estimate from a non-representative Belgian adult sample (mean = 15 ng/kg bw/day) (Geens et al., 2010). Nevertheless, as observed for the adolescent sample, the Chinese Total Diet Study (Yao et al., 2020) and EFSA Scientific Opinion (European Food Safety Authority, 2015) presented a dietary BPA exposure several times above the present results. The mean dietary exposure estimated for Chinese adults was 201.0 ng/kg bw/day (Yao et al., 2020). In Europe, the daily dietary BPA exposure of females, aged between 18 and 45 years old, was 132 ng/kg bw/day, while for males the exposure was 126 ng/kg bw/day (European Food Safety Authority, 2015).

Concerning the estimates calculated from urinary BPA applying random forest methodology, the results for the adolescent sample (26.7 ng/kg bw/day) and the adult Portuguese population (38.0 ng/kg bw/day) were in accordance with previous research. Most of the studies have made a direct backward calculation with urinary BPA concentration, not considering dietary variables and not adding uncertainty to the method, as it was done in this study using random forest and cross-validation, which limits a linear comparison of the results.

In 2017 a study of BPA estimates from global urinary concentration data was published, using information from 30 countries, including Portugal (Huang et al., 2017). The worldwide estimated BPA daily intake was 30.8 ng/kg bw/day in adults and 60.1 ng/kg bw/day in children. That publication used data from 20 adult participants from Portugal, estimating a BPA daily intake of approximately 36.0 ng/kg bw/day, very close to the one presented here. In a non-representative sample of children from Turkey, the geometric mean of BPA daily exposure was 35.0 ng/kg bw/day (Çok et al., 2020). In a non-representative sample of the general population from Korea, the BPA daily exposure was estimated as 23.0 ng/kg bw/day (Park et al., 2016). Finally, in a representative study from the United States, the median estimate of BPA daily intake using 2003–2004 NHANES urinary data was slightly higher than the estimates in this study: 50.5 ng/kg bw/day for the general population, 77.3 ng/kg bw/day for adolescents, 56.3 and 41.5 ng/kg bw/day for adults aged between 20 and 39 and 40 – 59 years, respectively (LaKind & Naiman, 2008).

Therefore, in our study, the random forest was the best methodological approach to estimate daily exposure to BPA. Recently, researchers also demonstrated that random forest performed better than linear regression in predicting serum vitamin D levels using intake data from food frequency questionnaires and lifestyle habits of Southern Europeans (Valer-Martinez et al., 2023). On the other hand, the validity of the food groups attribution is questionable. While the estimates align with some previous studies, this method may overestimate daily dietary exposure compared to urinary BPA levels. Additionally, all estimates of the three methodological approaches were above the TDI of 0.2 ng/kg body weight, which may indicate a public health concern for these adolescents and the adults of the Portuguese population.

The toxicological concern of BPA relies on its affinity to estrogenic receptors and, therefore, can interfere with the endocrine system's normal function, mimicking the effects of estrogenic hormones and modifying different signalling pathways (Gould et al., 1998; Maffini et al., 2006). In addition, the plausible mode of action of BPA, studied essentially in animal and *in vitro* models, indicated that this contaminant assigned in the development of metabolic syndrome, obesity, diabetes mellitus, cardiotoxicity, hyperuricemia, immunological disabilities, reproductive complications and neurodevelopmental/ neuroendocrine effects (Ballegaard & Bøgh, 2023; Bao et al., 2020; Fu et al., 2022; Maffini et al., 2006; Rochester, 2013; Shi et al., 2022; Yoo et al., 2020). Nevertheless, there are some uncertainties regarding the effects of BPA in humans that cannot be discarded due to the lack of epidemiological studies, especially with longitudinal designs (European Food Safety Authority, 2015; Vandenberg et al., 2007).

Another concern is the use of BPA analogues in their place due to the food legislation for this food contaminant (Meslin et al., 2022), which seems to have a similar mode of action to BPA once inside the organism (Liu et al., 2018; Rochester & Bolden, 2015).

The following steps should answer the uncertainties raised in the most recent authority's scientific opinions, longitudinally assessing the exposure of BPA, and their analogues, from childhood to adult life, and their association with health outcomes. This knowledge will help public health professionals and policymakers develop new and relevant health policies.

Some limitations of the present work must be considered in this discussion. First, the relatively small sample size of 24-h urine collection, especially in adults. However, this limitation was overcome by the type of biological samples collected, i.e. 24-h instead of a single spot (Gerofke et al., 2023; LaKind & Naiman, 2008; Meslin et al., 2022). Second, the possible bias associated with the collection of dietary

information, namely the participation bias in adolescents of 13 years or the possibility of misreporting in adults (Magalhães et al., 2020). Third, in adolescents, the food diary did not always correspond to the urine sample collected. Nevertheless, several non-consecutive days of dietary information may ameliorate this limitation. Fourth, the uncertainty in the data collected by adolescents regarding food packaging material may contribute to the difference between the results estimated by direct and indirect methods. Lastly, the interruption of the 13-year follow-up due to the COVID-19 pandemic affected the participation rate observed during this wave.

This study's main strength was applying direct and indirect methods to estimate the exposure to BPA, developing three different methodological approaches and identifying the most appropriate. This study proved that by applying the random forest, even with a limited number of urine samples, it was possible to estimate BPA daily exposure for the remaining sample/population. This methodological approach can now be used to estimate the daily exposure to other food contaminants, possibly testing the combined effect of different xenobiotic compounds. Another relevant strength was the availability of data on food consumption and BPA measured in urine samples from a cross-sectional sample of a prospective population-based birth cohort and a representative sample of the adult Portuguese population. The detail of the collected information was achieved by following standardized European methodology (European Food Safety Authority, 2014), especially related to the packaging material. Lastly, the daily dietary exposure to BPA was weighted for the complex survey design assuring national representativeness for adults.

The present study highlights the need to reduce dietary exposure to BPA, since chronic exposure safe levels are exceeded and adverse health effects are probable. For that, consumers should reduce the consumption of canned food and beverages and/or choose less contaminated food items, for example, fresh and unpackaged products.

5. Conclusion

The estimated daily exposure to BPA strongly changed depending on the methodological approach. According to the concentration of BPA measured in urine samples, the food groups attribution may overestimate the daily exposure to this food contaminant while the random forest was the most suitable methodology to estimate the daily exposure to BPA. Nevertheless, whatever the method used, adolescents at 13 years old and the adult Portuguese population presented an unsafe level of daily exposure to BPA, largely exceeding the safe levels recently proposed by EFSA.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendices.

See Figs. A1-A5.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.113251.

References

Adeyi, A. A., & Babalola, B. A. (2019). Bisphenol-A (BPA) in Foods commonly consumed in Southwest Nigeria and its Human Health Risk. *Scientific Reports, 9*(1), 17458. https://doi.org/10.1038/s41598-019-53790-2

Ballegaard, A. S. R., & Bøgh, K. L. (2023). Intestinal protein uptake and IgE-mediated food allergy. In *Food research international (Ottawa, Ont.)* (Vol. 163, p. 112150). NLM (Medline). https://doi.org/10.1016/j.foodres.2022.112150.

Bao, W., Liu, B., Rong, S., Dai, S. Y., Trasande, L., & Lehmler, H. J. (2020). Association Between Bisphenol A Exposure and Risk of All-Cause and Cause-Specific Mortality in US Adults. *JAMA Network Open, 3*(8), e2011620.

Beckmann, M., Lloyd, A. J., Wilson, T., Torres, D. P. M., Goios, A. C. L., Willis, N. D., Lyons, L., Phillips, H., Mathers, J. C., Nash, R. J., Sharp, H., & Draper, J. (2020). Calystegines are Potential Urine Biomarkers for Dietary Exposure to Potato Products. *Molecular Nutrition and Food Research, 64*(20), 1–11. https://doi.org/10.1002/mnfr.202000515

Bemrah, N., Jean, J., Rivière, G., Sanaa, M., Leconte, S., Bachelot, M., Deceuninck, Y., Bizec, B. L., Dauchy, X., Roudot, A.-C., Camel, V., Grob, K., Feidt, C., Picard-Hagen, N., Badot, P.-M., Foures, F., & Leblanc, J.-C. (2014). Assessment of dietary exposure to bisphenol A in the French population with a special focus on risk characterisation for pregnant French women. *Food and Chemical Toxicology, 72*, 90–97. https://doi.org/10.1016/j.fct.2014.07.005

Bingham, S. A. (2002). Biomarkers in nutritional epidemiology. *Public Health Nutrition, 5*(6a), 821–827. https://doi.org/10.1079/PHN2002368

Brouwer-Brolsma, E. M., Brennan, L., Drevon, C. A., Van Kranen, H., Manach, C., Dragsted, L. O., … Feskens, E. J. M. (2017). *Combining traditional dietary assessment methods with novel metabolomics techniques: Present efforts by the Food Biomarker Alliance, 76*, 619–627.

Cacho, J. I., Campillo, N., Viñas, P., & Hernández-Córdoba, M. (2012). Stir bar sorptive extraction coupled to gas chromatography–mass spectrometry for the determination of bisphenols in canned beverages and filling liquids of canned vegetables. *Journal of Chromatography A, 1247*, 146–153. https://doi.org/10.1016/j.chroma.2012.05.064

Chang, W. H., Liu, S. C., Chen, H. L., & Lee, C. C. (2019). Dietary intake of 4-nonylphenol and bisphenol A in Taiwanese population: Integrated risk assessment based on probabilistic and sensitive approach. *Environmental Pollution, 244*, 143–152. https://doi.org/10.1016/J.ENVPOL.2018.10.040

Çok, İ., İkidağ, Ö. T., Battal, D., & Aktaş, A. (2020). Assessment of bisphenol a levels in preschool children: Results of a human biomonitoring study in ankara, turkey. *JCRPE Journal of Clinical Research in Pediatric Endocrinology, 12*(1), 86–94. https://doi.org/10.4274/jcrpe.galenos.2019.2019.0087

Costa, S. A., Vilela, S., Correia, D., Severo, M., Lopes, C., & Torres, D. (2021). Consumption of packaged foods by the Portuguese population: Type of materials and its associated factors. *British Food Journal, 123*(2), 833–846. https://doi.org/10.1108/BFJ-07-2020-0584

Cousins, I. T., Staples, C. A., Klečka, G. M., & Mackay, D. (2002). A Multimedia Assessment of the Environmental Fate of Bisphenol A. *Human and Ecological Risk Assessment: An International Journal, 8*(5), 1107–1135. https://doi.org/10.1080/1080-700291905846

Cunha, S. C., & Fernandes, J. O. (2010). Quantification of free and total bisphenol A and bisphenol B in human urine by dispersive liquid-liquid microextraction (DLLME) and heart-cutting multidimensional gas chromatography-mass spectrometry (MD-GC/MS). *Talanta, 83*(1), 117–125. https://doi.org/10.1016/j.talanta.2010.08.048

de Fátima Poças, M., & Hogg, T. (2007). Exposure assessment of chemicals from packaging materials in foods: a review. In *Trends in Food Science and Technology* (Vol. 18, Issue 4, pp. 219–230). https://doi.org/10.1016/j.tifs.2006.12.008.

Commission, E. (2011a). Commission Implementing Regulation (EU) No 321/2011 of 1 April 2011 amending Regulation (EU) No 10/2011 as regards the restriction of use of Bisphenol A in plastic infant feeding bottles. *Official Journal of the European Union, L, 87*, 1–2. http://data.europa.eu/eli/reg_impl/2011/321/oj.

Commission, E. (2011b). Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. *Official Journal of the European Union, L, 12*, 1–89. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:012:0001:0089:EN:PDF.

Commission, E. (2018). Commission Regulation (EU) No 2018/213 of 12 February 2018 on the use of bisphenol A in varnishes and coatings intended to come into contact with food and amending Regulation (EU) No 10/2011 as regards the use of that substance in plastic food contact mat. *Official Journal of the European Union, L, 41*, 6–12. http://data.europa.eu/eli/reg/2018/213/oj.

European Food Safety Authority. (2011). Report on the development of a Food Classification and Description System for exposure assessment and guidance on its implementation and use. *EFSA Journal, 9*(12), 1–84. https://doi.org/10.2903/j.efsa.2011.2489.

European Food Safety Authority. (2014). Guidance on the EU Menu methodology. *EFSA Journal, 12*(12). https://doi.org/10.2903/j.efsa.2014.3944.

European Food Safety Authority. (2015). Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. *EFSA Journal, 13*(1). https://doi.org/10.2903/j.efsa.2015.3978.

European Food Safety Authority. (2023). Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. *EFSA Journal, 21*(4), e06857. https://doi.org/https://doi.org/10.2903/j.efsa.2023.6857.

European Food Safety Authority Panel on Food Contact Materials Enzymes and Processing Aids. (2021). *Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs*.

Fu, X., He, J., Zheng, D., Yang, X., Wang, P., Tuo, F., Wang, L., Li, S., Xu, J., & Yu, J. (2022). Association of endocrine disrupting chemicals levels in serum, environmental risk factors, and hepatic function among 5- to 14-year-old children. *Toxicology, 465*, Article 153011. https://doi.org/10.1016/j.tox.2021.153011

Geens, T., Apelbaum, T. Z., Goeyens, L., Neels, H., & Covaci, A. (2010). Intake of bisphenol A from canned beverages and foods on the Belgian market. *Food Additives and Contaminants - Part A, 27*(11), 1627–1637. https://doi.org/10.1080/19440049.2010.508183

Gerofke, A., David, M., Schmidt, P., Vicente, J. L., Buekers, J., Gilles, L., … Kolossa-Gehring, M. (2023). From science to policy: How European HBM indicators help to answer policy questions related to phthalates and DINCH exposure. *International Journal of Hygiene and Environmental Health, 247*, Article 114073. https://doi.org/10.1016/J.IJHEH.2022.114073

Goios, A. C. L., Severo, M., Lloyd, A. J., Magalhães, V. P. L., Lopes, C., & Torres, D. P. M. (2020). Validation of a new software eAT24 used to assess dietary intake in the adult Portuguese population. *Public Health Nutrition, 3*, 1–11. https://doi.org/10.1017/S1368980020001044

Gorecki, S., Bemrah, N., Roudot, A.-C., Marchioni, E., Le Bizec, B., Faivre, F., Kadawathagedara, M., Botton, J., & Rivière, G. (2017). Human health risks related to the consumption of foodstuffs of animal origin contaminated by bisphenol A. *Food and Chemical Toxicology, 110*, 333–339. https://doi.org/10.1016/j.fct.2017.10.045

Gould, J. C., Leonard, L. S., Maness, S. C., Wagner, B. L., Conner, K., Zacharewski, T., Safe, S., McDonnell, D. P., & Gaido, K. W. (1998). Bisphenol A interacts with the estrogen receptor α in a distinct manner from estradiol. *Molecular and Cellular Endocrinology, 142*(1), 203–214. https://doi.org/10.1016/S0303-7207(98)00084-7

Guerra, R. S., Fonseca, I., Pichel, F., Restivo, M. T., & Amaral, T. F. (2014). Hand length as an alternative measurement of height. *European Journal of Clinical Nutrition, 68*(2), 229–233. https://doi.org/10.1038/ejcn.2013.220

Hansch, C., Leo, A., & Heller, S. R. (1995). *Exploring QSAR - hydrophobic, electronic, and steric constants*. American Chemical Society.

Huang, R. ping, Liu, Z. hua, Yuan, S. fen, Yin, H., Dang, Z., & Wu, P. xiao. (2017). Worldwide human daily intakes of bisphenol A (BPA) estimated from global urinary concentration data (2000–2016) and its risk analysis. *Environmental Pollution, 230*, 143–152. https://doi.org/10.1016/j.envpol.2017.06.026.

Kang, J.-H., Kondo, F., & Katayama, Y. (2006). Human exposure to bisphenol A. *Toxicology, 226*(2), 79–89. https://doi.org/10.1016/j.tox.2006.06.009

Koch, H. M., Aylward, L. L., Hays, S. M., Smolders, R., Moos, R. K., Cocker, J., Jones, K., Warren, N., Levy, L., & Bevan, R. (2014). Inter- and intra-individual variation in urinary biomarker concentrations over a 6-day sampling period. Part 2: Personal care product ingredients. *Toxicology Letters, 231*(2), 261–269. https://doi.org/10.1016/j.toxlet.2014.06.023

LaKind, J. S., & Naiman, D. Q. (2008). Bisphenol A (BPA) daily intakes in the United States: Estimates from the 2003–2004 NHANES urinary BPA data. *Journal of Exposure Science and Environmental Epidemiology, 18*(6), 608–615. https://doi.org/10.1038/jes.2008.20

Lakind, J. S., & Naiman, D. Q. (2011). Daily intake of bisphenol A and potential sources of exposure: 2005–2006 National Health and Nutrition Examination Survey. *Journal of Exposure Science & Environmental Epidemiology, 21*(3), 272–279. https://doi.org/10.1038/jes.2010.9

Larsen, P. S., Kamper-Jørgensen, M., Adamson, A., Barros, H., Bonde, J. P., Brescianini, S., Brophy, S., Casas, M., Devereux, G., Eggesbø, M., Fantini, M. P., Frey, U., Gehring, U., Grazuleviciene, R., Henriksen, T. B., Hertz-Picciotto, I., Heude, B., Hryhorczuk, D. O., Inskip, H., … Andersen, A. M. N. (2013). Pregnancy and birth cohort resources in Europe: A large opportunity for aetiological child health research. In *Paediatric and Perinatal Epidemiology* (Vol. 27, Issue 4, pp. 393–414). Blackwell Publishing Ltd. https://doi.org/10.1111/ppe.12060.

Lide, D. R., & ed. (2005). *CRC Handbook of Chemistry and Physics* (95th ed.). CRC Press.

Liu, J., Wattar, N., Field, C. J., Dinu, I., Dewey, D., & Martin, J. W. (2018). Exposure and dietary sources of bisphenol A (BPA) and BPA-alternatives among mothers in the APrON cohort study. *Environment International, 119*(March), 319–326. https://doi.org/10.1016/j.envint.2018.07.001

Lopes, C., Torres, D., Oliveira, A., Severo, M., Guiomar, S., Alarcão, V., Ramos, E., Rodrigues, S., Vilela, S., Oliveira, L., Mota, J., Teixeira, P. J., Nicola, P. J., Soares, S., & Andersen, L. F. (2018). National food, nutrition, and physical activity survey of the Portuguese general population (2015–2016): Protocol for design and development. *Journal of Medical Internet Research, 20*(2), 1–11. https://doi.org/10.2196/resprot.8990

Lopes, C., Torres, D., Oliveira, A., Severo, M., Guiomar, S., Alarcão, V., Vilela, S., Ramos, E., Rodrigues, S., Oliveira, L., Nicola, P., Mota, J., Teixeira, P., & Soares, S. (2017). National Food, Nutrition and Physical Activity Survey of the Portuguese general population. *EFSA Supporting Publications, 14*(12). https://doi.org/10.2903/sp.efsa.2017.en-1341

Maffini, M. V., Rubin, B. S., Sonnenschein, C., & Soto, A. M. (2006). Endocrine disruptors and reproductive health: The case of bisphenol-A. *Molecular and Cellular Endocrinology, 254–255*, 179–186. https://doi.org/10.1016/J.MCE.2006.04.033

Magalhães, V., Severo, M., Torres, D., Ramos, E., & Lopes, C. (2020). Characterizing energy intake misreporting and its effects on intake estimations, in the Portuguese adult population. *Public Health Nutrition, 23*(6), 1031–1040. https://doi.org/10.1017/S1368980019002465

Meslin, M., Beausoleil, C., Zeman, F. A., Antignac, J. P., Kolossa-Gehring, M., Rousselle, C., & Apel, P. (2022). Human Biomonitoring Guidance Values (HBM-GVs) for Bisphenol S and Assessment of the Risk Due to the Exposure to Bisphenols A and S, in Europe. *Toxics 2022, Vol. 10, Page 228, 10*(5), 228. https://doi.org/10.3390/TOXICS10050228.

Noureddine El Moussawi, S., Cladière, M., Chébib, H., Ouaini, R., & Camel, V. (2019). Empirical models to predict the effect of sterilisation and storage on bisphenols migration from metallic can coatings into food simulants. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk. Assessment, 36*(12), 1937–1949. https://doi.org/10.1080/19440049.2019.1670870

Noureddine El Moussawi, S., Ouaini, R., Matta, J., Chébib, H., Cladière, M., & Camel, V. (2019). Simultaneous migration of bisphenol compounds and trace metals in canned vegetable food. *Food Chemistry, 288*, 228–238. https://doi.org/10.1016/j.foodchem.2019.02.116

Olea, N., Pulgar, R., Pérez, P., Olea-Serrano, F., Rivas, A., Novillo-Fertrell, A., Pedraza, V., Soto, A. M., & Sonnenschein, C. (1996). Estrogenicity of resin-based composites and sealants used in dentistry. *Environmental Health Perspectives, 104*(3), 298–305. https://doi.org/10.1289/ehp.96104298

Oliveira, A., Araújo, J., Severo, M., Correia, D., Ramos, E., Torres, D., Lopes, C., Rodrigues, S., Vilela, S., Guiomar, S., Oliveira, L., Alarcão, V., Nicola, P., Mota, J., Teixeira, P., Soares, S., & Andersen, L. F. (2018). Prevalence of general and abdominal obesity in Portugal: Comprehensive results from the National Food, nutrition and physical activity survey 2015–2016. *BMC Public Health, 18*(1), 1–9. https://doi.org/10.1186/s12889-018-5480-z

Park, J. H., Hwang, M. S., Ko, A., Jeong, D. H., Lee, J. M., Moon, G., Lee, K. S., Kho, Y. H., Shin, M. K., Lee, H. S., Kang, H. S., Suh, J. H., & Hwang, I. G. (2016). Risk assessment based on urinary bisphenol A levels in the general Korean population. *Environmental Research, 150*, 606–615. https://doi.org/10.1016/j.envres.2016.03.024

Pollack, A. Z., Perkins, N. J., Sjaarda, L., Mumford, S. L., Kannan, K., Philippat, C., Wactawski-Wende, J., & Schisterman, E. F. (2016). Variability and exposure classification of urinary phenol and paraben metabolite concentrations in reproductive-aged women. *Environmental Research, 151*, 513–520. https://doi.org/10.1016/j.envres.2016.08.016

Pulgar, R., Olea-Serrano, M. F., Novillo-Fertrell, A., Rivas, A., Pazos, P., Pedraza, V., Navajas, J. M., & Olea, N. (2000). Determination of bisphenol A and related aromatic compounds released from bis-GMA-based composites and sealants by high performance liquid chromatography. *Environmental Health Perspectives, 108*(1), 21–27. https://doi.org/10.1289/ehp.0010821

Rochester, J. R. (2013). Bisphenol A and human health: A review of the literature. *In Reproductive Toxicology (Vol., 42*, 132–155. https://doi.org/10.1016/j.reprotox.2013.08.008

Rochester, J. R., & Bolden, A. L. (2015). Bisphenol S and F: A Systematic Review and Comparison of the Hormonal Activity of Bisphenol A Substitutes. *Environmental Health Perspectives, 123*(7), 643. https://doi.org/10.1289/EHP.1408989

Rudel, R. A., Gray, J. M., Engel, C. L., Rawsthorne, T. W., Dodson, R. E., Ackerman, J. M., Rizzo, J., Nudelman, J. L., & Brody, J. G. (2011). Food packaging and bisphenol A and bis(2-ethyhexyl) phthalate exposure: Findings from a dietary intervention. *Environmental Health Perspectives, 119*(7), 914–920. https://doi.org/10.1289/ehp.1003170

Sakhi, A. K., Lillegaard, I. T. L., Voorspoels, S., Carlsen, M. H., Løken, E. B., Brantsæter, A. L., Haugen, M., Meltzer, H. M., & Thomsen, C. (2014). Concentrations of phthalates and bisphenol A in Norwegian foods and beverages and estimated dietary exposure in adults. *Environment International, 73*, 259–269. https://doi.org/10.1016/J.ENVINT.2014.08.005

Schecter, A., Malik, N., Haffner, D., Smith, S., Harris, T. R., Paepke, O., & Birnbaum, L. (2010). Bisphenol A (BPA) in U.S. food. *Environmental Science & Technology, 44*(24), 9425–9430. https://doi.org/10.1021/es102785d

Shi, Y., Wang, H., Zhu, Z., Ye, Q., Lin, F., & Cai, G. (2022). Association between exposure to phenols and parabens and cognitive function in older adults in the United States: A cross-sectional study. *Science of The Total Environment, 160129*. https://doi.org/10.1016/j.scitotenv.2022.160129

Stahlhut, R. W., Welshons, W. V., & Swan, S. H. (2009). Bisphenol A data in NHANES suggest longer than expected half-life, substantial nonfood exposure, or both. *Environmental Health Perspectives, 117*(5), 784–789. https://doi.org/10.1289/ehp.0800376

Sungur, Ş., Köroğlu, M., & Özkan, A. (2014). Determinatıon of bisphenol a migrating from canned food and beverages in markets. *Food Chemistry, 142*, 87–91. https://doi.org/10.1016/j.foodchem.2013.07.034

Thayer, K. A., Doerge, D. R., Hunt, D., Schurman, S. H., Twaddle, N. C., Churchwell, M. I., Garantziotis, S., Kissling, G. E., Easterling, M. R., Bucher, J. R., & Birnbaum, L. S. (2015). Pharmacokinetics of Bisphenol A in Humans Following a Single Oral Administration HHS Public Access. *Environ Int, 83*, 107–115. https://doi.org/10.1016/j.envint.2015.06.008

Torres, D., Faria, N., Sousa, N., Soares, R., Amorim, H., Guiomar, S., Lobato, L., Oliveira, C., Correia, D., Carvalho, C., Vilela, S., Severo, M., & Lopes, C. (2017). *Manual Fotográfico de Quantificação de Alimentos*. Universidade do Porto, 2017. www.ian-af.up.pt.

Valer-Martinez, A., Sayon-Orea, C., Martínez Hernandez, J. A., De la Fuente-Arrillaga, C., Pérez de Rojas, J., Barcones, F., Martínez-González, M. A., & Bes-Rastrollo, M. (2023). Forecasting levels of serum 25-hydroxyvitamin D based on dietary intake, lifestyle and personal determinants in a sample of Southern Europeans. *British Journal of Nutrition, 1–9*. https://doi.org/10.1017/S0007114523000946

Vandenberg, L. N., Hauser, R., Marcus, M., Olea, N., & Welshons, W. V. (2007). Human exposure to bisphenol A (BPA). *Reproductive Toxicology, 24*(2), 139–177. https://doi.org/10.1016/j.reprotox.2007.07.010

Von Goetz, N., Wormuth, M., Scheringer, M., & Hungerbühler, K. (2010). Bisphenol A: How the most relevant exposure sources contribute to total consumer exposure. *Risk Analysis, 30*(3), 473–487. https://doi.org/10.1111/j.1539-6924.2009.01345.x

Wang, C.-Y., Cogswell, M. E., Loria, C. M., Chen, T.-C., Pfeiffer, C. M., Swanson, C. A., Caldwell, K. L., Perrine, C. G., Carriquiry, A. L., Liu, K., Sempos, C. T., Gillespie, C. D., & Burt, V. L. (2013). Urinary excretion of sodium, potassium, and chloride, but not iodine, varies by timing of collection in a 24-hour calibration study. *The Journal of Nutrition, 143*(8), 1276–1282. https://doi.org/10.3945/jn.113.175927

WHO Multicentre Growth Reference Study Group. (2006). *Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age Methods and development.*

World Health Organization. (1984). *Estimation of Sodium Intake and Output: Review of Methods and Recommendations for Epidemiological Studies.*

Yao, K., Zhang, J., Yin, J., Zhao, Y., Shen, J., Jiang, H., & Shao, B. (2020). Bisphenol A and Its Analogues in Chinese Total Diets: Contaminated Levels and Risk Assessment. *Oxidative Medicine and Cellular Longevity, 2020*. https://doi.org/10.1155/2020/8822321

Yoo, S.-J., Joo, H., Kim, D., Lim, M. H., Kim, E., Ha, M., Kwon, H.-J., Paik, K. C., & Kim, K. M. (2020). Associations between exposure to bisphenol a and behavioral and cognitive function in children with attention-deficit/hyperactivity disorder: A case-control study. *Clinical Psychopharmacology and Neuroscience : The Official Scientific Journal of the Korean College of Neuropsychopharmacology, 18*(2), 261–269. https://doi.org/10.9758/cpn.2020.18.2.261

Paper III

Acrylamide daily dietary exposure and cardiometabolic outcomes: longitudinal approach from childhood to adolescence

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Submitted

Acrylamide daily dietary exposure and cardiometabolic outcomes: longitudinal approach from childhood to adolescence

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Abstract

Acrylamide is a food contaminant formed during the Maillard reaction that may act as an endocrine disruptor. The present study aims to assess acrylamide dietary exposure and test longitudinal associations with cardiometabolic outcomes from childhood to adolescence.

From G21-birth-cohort, 3138 participants with at least 2 consecutive follow-ups from 4 to 13 years of age were included. Acrylamide dietary exposure was estimated using 3-day food diaries and occurrence data of acrylamide in food.

Children presented a higher dietary acrylamide exposure than adolescents. The median exposure was, respectively, 0.79 µg/kg/d at 4 years and 0.44 at 13 years. Both children (MOE=215) and adolescents (MOE=386) exposure levels presented health concerns regarding neoplastic effects.

Cross-sectional and longitudinally, acrylamide dietary exposure was positively associated with blood glucose, waist circumference, and fat mass percentage. A negative association was found between dietary acrylamide exposure and z-BMI and cholesterol. This variability suggests that the effects of dietary acrylamide exposure on cardiometabolic health are complex and multifactorial. It was not possible to conclude, without uncertainty, that dietary acrylamide exposure is responsible for abnormalities in cardiometabolic outcomes from childhood to adolescence.

Keywords: cohort, food contaminants, risk assessment, children, adolescents, health

Introduction

The current management of non-communicable diseases presents a large public health challenge since their incidence, prevalence, and burden continue to increase in all countries (1). Cardiovascular diseases, cancer, diabetes and chronic respiratory diseases, as examples of non-communicable diseases, were responsible for three out of four deaths worldwide (2). In Portugal, despite the actions and interventions already implemented to reduce the progress, 87% of the deaths were attributed to non-communicable diseases, with an 11% probability of premature mortality because of these health conditions (2).

Cardiovascular diseases are associated with impaired blood pressure, blood glucose, insulin and lipids levels, and adiposity distribution in adults and children (3–5). These, along with other unhealthy lifestyle behaviours, such as unhealthy eating habits and physical inactivity, are key risk factors in the development of cardiometabolic disorders (6–10).

Usually, chronic diseases have a very low incidence in children and adolescents (11). Still, it is possible to identify abnormalities in the cardiometabolic profile in youth, which may negatively impact future health, decreasing the quality of life in adulthood (3,11,12).

Cooking and thermal processing foods are essential to ensure foodstuffs' safety, nutritional proprieties and desirable sensory characteristics, such as flavours, aromas, colours and texture (13,14). However, some non-intentional heat-induced hazardous substances are formed through the chemical reactions when the food is heated (14,15). Acrylamide is one of these hazardous compounds, identified in food products for the first time in 2002 by the Swedish Authorities, apart from their previous use/ occurrence in other non-food related applications (16). It is formed during the extension of the Maillard reaction at higher temperatures in low moisture conditions, through the reaction between asparagine amino acid and reducing sugars (17–19). Cereal products, potatoes, coffee and coffee substitutes are the main contributors to acrylamide exposure because of their higher concentration of carbohydrates and the associated industrial or home-cooking thermal methods, such as frying, baking, roasting and toasting (17,20,21). The diet culture of different geographical regions associated with varying patterns of eating and cooking habits, influence exposure to acrylamide (22–24). For the general non-smoker population, children and

adolescents, diet is the primary source of acrylamide. Still, other important routes of exposure could not be disclosed, namely dermal absorption or inhalation related to occupational settings and tobacco smoke (20,25).

After exposure and absorption, acrylamide is conjugated with glutathione or metabolised in glycidamide by cytochrome P450 enzyme complex, followed by its wide distribution into tissues (26,27). Acrylamide and glycidamide can form adducts by reacting with macromolecules, such as haemoglobin, DNA, serum albumin and enzymes, causing different toxicological effects: genotoxicity, carcinogenicity, neurotoxicity, hepatoxicity, cardiovascular toxicity and reproductive toxicity (20,28–30). In the last few years, some studies have shown that acrylamide may induce stress on the endocrine function, and it has been classified as a potential endocrine disruptor (31,32). Moreover, acrylamide plays an important role in oxidative stress, lipid peroxidation and adipogenesis, leading to abnormalities in serum lipid and glucose profiles (23,33,34). However, the mechanism through which acrylamide may cause cardiometabolic disturbances is not entirely understood or described. In fact, the evidence that clearly describes the association between this food contaminant and cardiometabolic outcomes is very limited, especially in children and adolescents. Most studies were performed in animal models or adults and focused on obesity and biochemical parameters of obesity-related comorbidities, showing controversial results (35–38). Additionally, most of these have cross-sectional designs, which does not allow the establishment of a causal association.

Despite the multifactorial causes related to the development of cardiometabolic disorders, exposure to food and environmental contaminants, such as acrylamide, in the early stages of life represents a risk factor for this health condition. Different authors concluded that there is a need for more epidemiological studies to fill the gap regarding the effect of acrylamide on human health, especially in children and adolescents (20,23,39).

Thus, the present study aims to perform exposure assessment and risk characterisation of dietary acrylamide and to longitudinally evaluate the association between this food contaminant exposure and cardiometabolic outcomes from childhood to adolescence.

Methods

Study design and participants

This project included data from Generation XXI (GXXI), an ongoing prospective population-based birth cohort assembled between April 2005 and August 2006 at the five public units providing obstetrical and neonatal care in the metropolitan area of Porto, Portugal, as previously described (40). Recruitment was conducted according to the following eligibility criteria: mothers living in one of the six municipalities of the metropolitan area of Porto; delivering at the public hospitals covering those municipalities; and giving birth, delivering at the public hospitals covering those municipalities, and giving birth to live babies with a gestational age of to live babies with gestational age 24 weeks. In the 24 to 72 hours after delivery, mothers were invited to participate in the cohort GXXI and 91% accepted to enrol, resulting in 8,495 mothers and 8,647 children. Subsequently, all participants were invited to participate in the 4-, 7-, 10- and 13-year follow-ups. The participation rates of each wave were 86%, 80%, 76% and 54%, respectively. The lower participation rate observed in the 13-year follow-up evaluation was related to the COVID-19 pandemic, which was prematurely interrupted in March 2020.

This study used data from all follow-up evaluations to perform a longitudinal approach. Eligible criteria were 1) providing a complete food diary (with at least two reported days) and 2) having at least two consecutive follow-ups with measured outcome variables. The flowchart of the included participants with the selection criteria information is described in Figure 1. From the baseline sample, 3368 were excluded due to the missing data on dietary intake in all follow-up evaluations. 7 participants presented an incomplete food diary, i.e., a food diary with less than two reported days. Finally, 2134 individuals were excluded, considering that they missed the criteria of being present in 2 consecutive follow-up evaluations, being 3138 the final sample of this study (Figure 1).

Sociodemographic characteristics, lifestyle factors and dietary intake

Sociodemographic characteristics were collected from the participants and mothers using standardised questionaries. Child sex, maternal age at baseline and maternal educational level (defined as the number of completed schooling years) were the variables considered, collected at baseline.

Maternal smoking habits and participants' physical activity were considered as lifestyle factors. At 4-years follow-up, mothers were asked about their smoking habits: current smokers, former smokers or never smoked. For this study, data from maternal smoking habits at 4-year follow-up were analysed as a potential confounder to acrylamide exposure. Physical activity was measured through the practice (yes/no) of some scheduled and regular sports activity outside the school setting at 4-, 7-, 10-, and 13-year follow-ups, asked the parents (or the primary caregiver) in the first three waves and to the adolescents at 13-years old.

Before the face-to-face interview, after receiving a set of oral and written instructions from the research team, parents (or the main caregiver) at 4-, 7- and 10-year follow-ups and adolescents at 13-year follow-up were invited to complete a 3-day food diary (two weekdays and one weekend day). For each food and beverage consumed, a substantial amount of detailed information was gathered, including consumption place and time, brand, food preparation method, cooking process and quantity consumed. During the face-to-face interview, the food diaries were reviewed by trained researchers, being codified into Food Processor SQL software (41), at 4-, 7- and 10-year follow-ups, and into "eAT24" software, at 13-year follow-up, by trained nutritionists. Dietary information from 4- to 10-years was harmonised in the "eAT24" software. Integrating the Portuguese Food Composition Table and adopting the FoodEx2 classification system, the "eAT24" software was developed for the National Food, Nutrition and Physical Activity Survey 2015-2016, as described previously (42,43). To be eligible in the present study, participants had to report at least two completed days in the food diary.

Anthropometrics measurements, blood pressure and cardiometabolic biomarkers

Height and weight were objectively measured during the face-to-face interviews, under standard procedures after 12 hours of fasting, with subjects in light clothing and barefoot (44). A wall stadiometer (SECA, Hamburg, Germany) was used to measure height to the nearest centimetre, and a digital scale (SECA, Columbia, USA) measured body weight to the nearest tenth of a kilogram. The body mass index (BMI) was computed through weight over the squared height, with the participants being classified according to age and sex-specific BMI z-scores (z-BMI) published by the World Health Organization (45).

With the participant in a standing position, relaxed abdomen, arms at the sides and feet together, waist circumference (WC) was measured at the umbilicus level to the nearest 0.1 centimetres (46,47).

According to standard procedures (48), a tetrapolar bioelectric impedance analysis was performed using BIA 101 Anniversary® (Akern, Florence, Italy), and the percentage of fat mass (%FM) was computed for all follow-up evaluations, applying the Schaefer et al. equation (49).

After a 10-minute rest, with an interval of at least 5 minutes, using an automatic upper arm blood pressure monitor (Omron®) placed over the brachial artery pulse, two measurements of systolic (SBP) and diastolic (DBP) blood pressure were taken, and their mean value calculated, at 4- and 7-year follow-ups. A third measurement was taken when the difference between the SBP and DBP was higher than 5 mmHg, considering the mean of the two closest values. At 10- and 13-year follow-ups, three measurements of SBP and DBP were taken, and the mean of the two closest values was calculated. Age-, sex- and height-specific z-scores for SBP and DBP (z-SBP and z-DBP) were calculated, following the American Academy of Pediatrics criteria (50).

For all participants who had consented, a blood sample was collected on the morning of the follow-up evaluation after a 12-hour overnight fast. Laboratory analyses were performed at the Clinical Pathology Department of the Hospital of São João, Porto, Portugal, for blood cardiometabolic biomarkers considered in this study. Glucose was measured using a UV enzymatic assay (hexokinase method), and insulin using an electrochemiluminescence immunoassay. Insulin resistance was computed according to the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) (51). Enzymatic colourimetric assays were used to measure triglycerides (TG), total cholesterol and high-density lipoprotein-cholesterol (HDL cholesterol). Finally, Friedewald et al. equation (52) was used to calculate low-density lipoprotein-cholesterol (LDL cholesterol).

Risk assessment of acrylamide exposure

A risk assessment was performed to evaluate the risk associated with acrylamide daily dietary exposure. After the identification and hazard characterisation of acrylamide, we assessed the exposure and characterised the risk in the present work.

For the exposure assessment, data on the occurrence of acrylamide in food groups were extracted from the EFSA Scientific Opinion on Acrylamide in Food, published in 2015 (20), and the daily dietary exposure to acrylamide (µg/kg of body weight/day) was calculated as previously described (21). For each food item reported, a value from the acrylamide distribution from the corresponding food group, published by EFSA, was randomly attributed: in 5% of the reports, the 95th percentile level was attributed; LOQ values were attributed to a fraction of the reports corresponding to the LC reported (upper-bound scenario); for the other reports, the calculated true mean of the remaining values of acrylamide concentration was attributed. Ten cycles of random attributions were performed, resulting in ten different estimated acrylamide values for each consumption occasion.

For risk characterisation, the lower confidence limits of the benchmark doses for a 10% extra risk ($BMDL_{10}$) for peripheral neuropathy (430 µg/kg of body weight/day) and neoplastic effects (170 µg/kg of body weight/day) were used to calculate the margins of exposure (MOE). MOEs lower than 125 and 10,000 indicate a health concern regarding non-genotoxic and genotoxic/ carcinogenic effects, respectively (20).

Ethics approval and consent to participate

All phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki (53). The baseline and follow-up evaluations were approved by the University of Porto Medical School/ S. João Hospital Centre Ethics Committee, except the 13-year follow-up that was approved by the ISPUP Ethics Committee. At baseline and follow-up evaluations, all procedures were explained to participants, and an informed consent was signed by one of the parents or legal guardians (at 13 years, it was also signed by the participants). The baseline evaluation was additionally approved by the Data Protection National Commission and the study follows the present EU General Data Protection Regulation under close supervision of the Data Protection Office of ISPUP.

Statistical analysis

A generalised least squares model was used to test the associations between acrylamide daily dietary exposure (µg/day) and cardiometabolic outcomes (Figure 2), presented by standardised linear regression coefficient (std β) and 95% confidence interval (95% CI). Total cholesterol, LDL cholesterol, HDL cholesterol, TG, glucose, insulin, HOMA-IR, z-SBP, z-DBP, z-BMI, %FM and WC were considered as cardiometabolic outcomes. Three regression models are presented in this paper: model 1 adjusted for cross-sectional and longitudinal acrylamide daily dietary exposure; model 2 adjusted for model 1 plus participants' sex, maternal age, maternal education, maternal smoking habits, practice of regular physical activity (cross-sectional and longitudinal); model 3 adjusted for model 2 plus total energy intake (kcal) (cross-sectional and longitudinal). Two additional models were conducted: model 4 adjusted for model 2 plus participants' height (m) (cross-sectional and longitudinal); model 5 adjusted for model 2 plus participants' weight (kg) (cross-sectional and longitudinal) (in appendices).

To assess the impact of including or excluding participants diagnosed with diabetes, cardiovascular disease, and renal disease on the associations, sensitivity analyses were performed.

Missing data on cardiometabolic biomarkers were treated at random. The R software version 4.2.1 for Windows was used, with a significance level of 5%.

Results

The comparison of sociodemographic characteristics between eligible participants and the remaining cohort at the baseline is presented in Table 1. Significant differences were found in all tested sociodemographic variables, where 52% of the individuals were males, born from mothers slightly older (30.8±4.9 vs 28.8±5.8, $p<0.001$) and more educated (11.6±4.3 vs 9.8±4.1, $p<0.001$).

The practice of leisure physical activity, cardiometabolic biomarkers and energy intake of eligible and non-eligible participants from 4- to 13 years are described in Table 2. No significant differences were found between eligible and non-eligible participants for most tested variables. A higher number of significant differences was found at 10-year follow-up (8 of 14 indicators) and a lower number at 13-year

follow-up (5 of 14). The effect size ranged between -0.15 and 0.14 (data not shown).

The exposure assessment and risk characterisation of acrylamide in eligible participants are described in Table 3. The daily median dietary exposure ranged from 0.44 to 0.79 µg/kg of body weight, and the 95th percentile from 1.04 to 1.92 at 13-years and 4-years follow-ups, respectively. The 4-years wave was the evaluation with the higher values of acrylamide exposure and, consequently, with the lower values of MOE across the entire distribution. The MOE of peripheral neuropathy calculated for the median point ranged between 544 (4-years) and 977 (13-years), whereas for the 95th percentile of acrylamide exposure ranged from 478 (4-years) to 878 (13-years). According to the current acrylamide daily dietary exposure, the MOE values for peripheral neuropathy were clearly above 125, which indicates no public health concern regarding non-genotoxic effects. The neoplastic effects MOE for median exposure was 215 at 4-years and 386 at 13-years, while for the 95th percentile of acrylamide exposure was 89 and 163, respectively. Even at the lower percentile of exposure (5th percentile), this MOE ranged between 708 (4-years) and 1214 (13-years). Thus, all estimated MOE for the neoplastic outcome were several times lower than 10,000, indicating a public health concern regarding genotoxic/ carcinogenic effects for the eligible participants of GXXI.

Table 4 presents the cross-sectional and longitudinal associations of acrylamide daily dietary exposure and cardiometabolic outcomes.

In the three models, after adjusting to all potential confounders, a cross-sectional and longitudinal negative significant association of acrylamide daily dietary exposure with z-BMI was found (model 3). According to the longitudinal exposure, each increased standard deviation of previous acrylamide exposure was statistically associated with a decrease of -0.07 (95% CI: -0.09, -0.04) in z-BMI (model 3).

In the three models, after adjusting to all potential confounders, a cross-sectional and longitudinal positive significant association of acrylamide daily dietary exposure with WC and blood glucose was found (model 3). According to the longitudinal exposure, each increased standard deviation of previous acrylamide exposure was statistically associated with an increase of 0.06 cm (95% CI: 0.03, 0.08) in WC and 0.03 mg/dL (95% CI: 0.00, 0.07) in blood glucose (model 3).

The percentage of fat mass was positively associated with both cross-sectional and longitudinal exposure to acrylamide, with statistical significance after the adjustment for sociodemographic characteristics, lifestyle variables, and energy intake. Longitudinally, for each standard deviation increase of previous acrylamide exposure, there is an increase of 0.04 mg/dL (95% CI: 0.01, 0.06) in the %FM (model 3).

A cross-sectional and longitudinal significant negative association between acrylamide daily dietary exposure and total cholesterol, LDL cholesterol, and HDL cholesterol was found in the three models. Longitudinally, for each standard deviation increase of previous acrylamide exposure, there is a decrease of -0.07 mg/dL (95% CI: -0.11, -0.04) on total cholesterol, -0.08 mg/dL (95% CI: -0.11, -0.04) on LDL cholesterol and -0.06 mg/dL (95% CI: -0.09, -0.02) on HDL cholesterol, in the subsequent follow-up (model 3). No significant associations were found for triglyceride serum levels in any model.

Blood insulin and HOMA-IR were statistically positively associated with cross-sectional and longitudinal exposure to acrylamide in models 1 and 2. However, after adjusting for total energy intake, the longitudinal exposure lost significance (model 3). Cross-sectionally, for each increase in the standard deviation of acrylamide exposure, there is an increase of 0.08 (95% CI: 0.05, 0.12) in blood insulin and HOMA-IR (model 3).

After adjusting for all confounders, a negative statistically significant longitudinal association was found between acrylamide dietary daily exposure and z-SBP. For z-DBP, a significant negative association was found for both cross-sectional and longitudinal exposure after adjustment for all confounding variables.

An additional analysis was performed to test the association of acrylamide daily dietary exposure on cardiometabolic outcomes, including two models adjusted for participants' weight and height (appendices, Table S1, models 4 and 5). We found significant associations for most of the health outcomes that also showed significant associations in model 3; however, these associations were in the opposite direction.

A significant positive association was found between cross-sectional and longitudinal acrylamide dietary exposure and total cholesterol, LDL cholesterol and HDL cholesterol after the adjustment for sociodemographic characteristics, lifestyle variables, and participants' weight (Table S1, model 4). However, these

significances were lost after adjusting for sociodemographic characteristics, lifestyle variables, and participants' height, except for the association between cross-sectional acrylamide exposure and HDL cholesterol (Table S1, model 5).

A significant negative association was found between acrylamide exposure and WC, %FM, blood insulin, and HOMA-IR, for both cross-sectional and longitudinal exposure, after adjusting for participants' weight and participants' height (Table S1, models 4 and 5).

Blood glucose showed a significant negative cross-sectional and longitudinal association with acrylamide dietary exposure after adjusting for participants' weight (Table S1, model 4) and a significant positive cross-sectional association after adjusting for participants' height (Table S1, model 5).

According to the r-squared (Table 4 and Table S1), the proportion of variance explained by acrylamide daily dietary exposure was low. Finally, excluding participants diagnosed with diabetes, cardiovascular disease, and renal disease did not substantially change our findings (data not shown).

Discussion

To our knowledge, this is the first prospective study assessing the effects of acrylamide daily dietary exposure on cardiometabolic outcomes from childhood to adolescence.

Acrylamide dietary exposure was associated with several adverse health outcomes, namely cholesterol, blood glucose, insulin resistance, anthropometric measures, and blood pressure, impairing future stages of life from childhood to adolescence. However, the interpretation of the results should be made carefully, and the choice of the final model depends on the specific question we aim to address. Considering the conceptual framework of the present study and the possible effect of acrylamide as an endocrine disruptor, our final model included the adjustment for sociodemographic characteristics, lifestyle variables, and total energy intake. On the one hand, according to our hypothesis, participants' body weight may mediate the effect of the association. Because of that, the correct model should not be adjusted for this variable. This perspective was corroborated by the results found since the magnitude of the effect was attenuated, or some coefficients were reverted when considering the estimation of acrylamide adjusted for body weight. On the other hand, in our study, higher height was

correlated with higher energy consumption, so participants' height may be a proxy of total energy intake. Considering that, the plausibility of the effect of total energy intake on acrylamide exposure is higher than that of participants' height, the model adjusted for energy appeared to be the most appropriate to answer our objective.

Even so, within our final model, the results may seem slightly contradictory across the different cardiometabolic outcomes. We found a positive association between acrylamide exposure and waist circumference, fat mass percentage, higher blood glucose levels, blood insulin, and insulin resistance. In the opposite direction, a negative association was found for body mass index z-score, cholesterol, and blood pressure.

These variability in the results were aligned with previous studies, regardless of the difficulty of comparison according to the different study designs, sample sizes and methodologies. Across the available publications, the association with health outcomes was tested with the total exposure to acrylamide measured through blood biomarkers, such as haemoglobin adduct of acrylamide or haemoglobin adduct of glycidamide. Moreover, most of the studies were performed only on adults from the general population, which includes smokers and non-smokers. In this discussion, we will compare the results related to the non-smoker group or the model considering the smoking habit as a potential confounder.

In data from different cross-sectional evaluations of the NHANES study, considering only adults, an association between acrylamide exposure biomarker and body composition outcomes was found. However, looking deeply into the results, the same study found positive and negative associations or no significant effect, depending on the biomarker (54–56). In a cross-sectional study performed on 510 adults of an EPIC cohort, the authors found a positive association between acrylamide exposure and BMI in the non-smoker group, using glycidamide adduct and glycidamide-to-acrylamide adduct ratio but failed to find an association with acrylamide adducts (38). Contradictory results were also found for abdominal adiposity within and between studies (36,54). Two studies presented consistent results, showing no effect of acrylamide exposure on abdominal adiposity (37,57).

Regarding lipid profile, data from NHANES 2003-2004 showed no association between acrylamide exposure and total cholesterol, HDL cholesterol and

triglycerides in adults (57). In 2021, another study of NHANES considering cross-sectional moments from 2013 to 2016 concluded that there was a positive association with total cholesterol, LDL cholesterol and triglycerides, and a negative association with HDL cholesterol, despite the absence of significant effects for some outcomes according to the exposure biomarker (58). The same conclusions regarding HDL-cholesterol and triglycerides were obtained in a different study of adults, considering NHANES 2003-2006 and 2013-2016 information (36). On the contrary, another research group found an inverse association of acrylamide-haemoglobin adducts with higher triglycerides and low HDL cholesterol levels but failed to find an association with glycidamide-haemoglobin adducts (37).

For glucose homeostasis, according to our results, a cross-sectional study showed a clear positive correlation between acrylamide exposure and higher fasting plasma glucose levels in 3270 adults from a Chinese community-based cohort (35). Nevertheless, inconsistent results were found in two different studies, according to the biomarkers used to estimate acrylamide exposure (36,37). One cross-sectional study found an inverse association between acrylamide biomarkers and blood insulin or insulin resistance. Still, it failed to show an association with blood glucose and the remaining outcomes considering glycidamide biomarkers as exposure (57). Finally, data from NHANES 2003-2006 and 2013-2016 found inconsistent results for type 2 diabetes and hypertension according to the exposure biomarker (56).

It is important to highlight that chronic diseases, such as obesity, metabolic syndrome or abnormalities in cardiometabolic health, have multifactorial causes comprising energy imbalance and exposure to environmental and food contaminants (23,37). Moreover, these chemicals, namely endocrine disruptors, may present a U-shaped dose-response curve for some cardiometabolic outcomes (56,59), which may explain the controversial results found within and between studies. From another perspective, the synergic or protective effect from other diet components and other food contaminants cannot be disclosed (37,60), which increases the uncertainty of the results observed across previous publications and enforces the complexity of human health and diet.

The effect of acrylamide on genotoxicity and carcinogenicity has been amply demonstrated (20), being the most concerning health effect associated with

acrylamide exposure. In the last years, it has been suggested that modifications in cardiometabolic profiles may be caused by oxidative stress resulting from exposure to acrylamide (35,61). However, the results of this study do not allow us to conclude without uncertainty that acrylamide exposure, by itself, is responsible for coherent abnormalities in cardiometabolic outcomes from childhood to adolescence.

The estimated daily dietary exposure to acrylamide was in line with the results previously found for children and adolescents in the Portuguese population (21). Data from the National Food, Nutrition and Physical Activity Survey 2015-2016 showed that children and adolescents were the groups with higher acrylamide exposure and higher health concerns regarding neoplastic effects, with a median (P95) of exposure of 0.69 (1.32) and 0.54 (1.07) µg/kg/day in the age groups 3-9 years and 10-17 years, respectively. Moreover, despite common methodological differences, the results observed in the presented study were in accordance with other worldwide studies (20,60,62,63).

Even so, the exposure to acrylamide increased until 2017 as a reflection of changes in the population's dietary patterns (24), where the consumption of coffee, fried potatoes, and cereal-based products (bread, breakfast cereals, and cookies) is believed to have been intensified. Different authorities and research groups already published mitigation measures and benchmark levels to reduce the presence of acrylamide in food (28,30,64). Thus, from the public health perspective and following the precaution principle, combined efforts to decrease acrylamide exposure should be taken, especially at younger ages. Likewise, more studies are needed to investigate the association of acrylamide exposure with carcinogenic and non-carcinogenic health outcomes from childhood to adulthood, as well as the impact of mitigation strategies on the burden of diseases.

Limitations and strengths

Some limitations must be considered. First, the healthier characteristics of the eligible participants suggest a possible participation bias. However, considering the sample size and the effect size, the clinical relevance of these differences may be arguable. Second, using the upper-bound value for left-censored data may overestimate the acrylamide exposure assessment. Nevertheless, our

research group followed a more conservative approach, assessing the worst-case scenario. Third, differences in acrylamide occurrence, as a consequence of ingredients composition, cooking methods and cooking extension, either in industry or at home, were not considered. Fourth, the effect of acrylamide, by itself, on multifactorial cardiometabolic outcomes may be reductionist, as previously mentioned. Finally, the time frame of this study may not be sufficient to observe modifications in cardiometabolic profiles in children and adolescents.

This study also has several strengths. First, extensive information was collected on sociodemographic characteristics, food consumption and cardiometabolic biomarkers from a prospective population-based birth cohort. Second, the large sample size of eligible participants. Third, variability on acrylamide occurrence in foods was included, extracting the maximum information from the EFSA publication (20). Fourth, the performance of 10 cycles of random attributions increases the inter-individual and intra-individual variability. Finally, the longitudinal models were adjusted for several relevant confounders, including maternal smoking habits, leisure physical activity, participants' total energy intake, height and weight, representing another strength of this study.

Conclusion

This was the first study performed at younger ages, testing the association of acrylamide exposure on cardiometabolic outcomes, from childhood to adolescence. Comprehensive data from the Portuguese population-based birth cohort Generation XXI was used. Compared to adolescents, children presented a higher acrylamide exposure. Both children (MOE 215) and adolescents (MOE 386) exposure levels presented health concerns regarding neoplastic effects.

Acrylamide dietary exposure was associated with several health outcomes, particularly those more related to blood glucose, insulin resistance, and central and total body fat, but not with body mass index. On the contrary, it was negatively associated with z-BMI, as well as cholesterol lipid profiles and blood pressure. Nonetheless, the interpretation of the results should be made with caution. This variability in results suggests that the effects of acrylamide on cardiometabolic health are complex and may be influenced by multiple factors. Thus, it is not possible to conclude, without uncertainty, that acrylamide exposure alone is

responsible for observed abnormalities in cardiometabolic outcomes from childhood to adolescence.

Despite the limitations in establishing a clear causal relationship between acrylamide exposure and cardiometabolic outcomes, both children and adolescents presented very low margins of exposure to acrylamide regarding neoplastic effects. This underscores the importance of implementing food policies and guidelines aimed at reducing acrylamide exposure in younger populations. Future research is needed to explore further the long-term health effects of acrylamide exposure from childhood to adulthood and assess the mitigation strategies' effectiveness in reducing the burden of associated diseases.

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Data availability

The data from Generation XXI are not publicly available due to privacy or ethical restrictions. The data can be made available for research proposals on request to the Generation XXI Executive Committee (generationxxi@ispup.up.pt). Further information about Generation XXI can be obtained via the Generation XXI website [www.geracao21.com] or by emailing generationxxi@ispup.up.pt.

Disclosure statement

The authors have no competing interests to declare.

Contribute of authors

SAC, CL and DT contributed to the design and implementation of the research. MS and SAC to the analysis of the results.

SAC wrote the original draft manuscript. SAC, MS, CL and DT reviewed the manuscript.

References

1. James SL, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet [Internet]. 2018 Nov 10;392(10159):1789–858. Available from: https://doi.org/10.1016/S0140-6736(18)32279-7
2. World Health Organization. Noncommunicable diseases progress monitor 2022 [Internet]. 2022. Available from: https://apps.who.int/iris/bitstream/handle/10665/353048/9789240047761-eng.pdf
3. Singleton CMH, Brar S, Robertson N, DiTommaso L, Fuchs GJ, Schadler A, et al. Cardiometabolic risk factors in South American children: A systematic review and meta-analysis [Internet]. Vol. 18, PloS one. 2023. e0293865 p. Available from: http://dx.doi.org/10.1371/journal.pone.0293865
4. Kim M, Kim J. Cardiometabolic risk factors and metabolic syndrome based on severity of obesity in Korean children and adolescents: data from the Korea National Health and Nutrition Examination Survey 2007–2018. Ann Pediatr Endocrinol Metab. 2022;27(4):289–99.
5. Lassale C, Tzoulaki I, Moons KGM, Sweeting M. Europe PMC Funders Group Separate and combined associations of obesity and metabolic health with coronary heart disease : a pan-European case-cohort analysis. 2018;39(5):1–19.
6. García-Hermoso A, Ramírez-Vélez R, García-Alonso Y, Alonso-Martínez AM, Izquierdo M. Association of Cardiorespiratory Fitness Levels During Youth With Health Risk Later in Life: A Systematic Review and Meta-analysis. JAMA Pediatr. 2020 Oct;174(10):952–60.
7. Palhares HM da C, da Silva AP, Resende DCS, Pereira G de A, Rodrigues VJ, Borges M de F. Evaluation of clinical and laboratory markers of cardiometabolic risk in overweight and obese children and adolescents. Clinics (Sao Paulo). 2017 Jan;72(1):36–43.
8. Filho NS, Reuter CP, Silveira JFDC, Borfe L, Renner JDP, Pohl HH. Low performance-related physical fitness levels are associated with clustered cardiometabolic risk score in schoolchildren: a cross-sectional study. Human

Movement [Internet]. 2022;23(3):113–9. Available from: https://doi.org/10.5114/hm.2022.107976

9. Gonçalves C, Bravo J, Pais J, Abreu A, Raimundo A. Improving Health Outcomes in Coronary Artery Disease Patients with Short-Term Protocols of High-Intensity Interval Training and Moderate-Intensity Continuous Training: A Community-Based Randomized Controlled Trial. Cardiovasc Ther. 2023;2023:6297302.
10. Heinl RE, Dhindsa DS, Mahlof EN, Schultz WM, Ricketts JC, Varghese T, et al. Comprehensive Cardiovascular Risk Reduction and Cardiac Rehabilitation in Diabetes and the Metabolic Syndrome. Can J Cardiol. 2016 Oct;32(10 Suppl 2):S349–57.
11. de Castro Silveira JF, Sehn AP, da Silva L, Gaya AR, Lima RA, Burns RD, et al. The stability of cardiometabolic risk factors clustering in children and adolescents: a 2-year longitudinal study. J Diabetes Metab Disord [Internet]. 2023;22(1):529–38. Available from: https://doi.org/10.1007/s40200-022-01174-1
12. Fonseca MJ, Moreira C, Santos AC. Adiposity rebound and cardiometabolic health in childhood: Results from the Generation XXI birth cohort. Int J Epidemiol. 2021;50(4):1260–71.
13. Tucker GS, Featherstone S. Essentials of Thermal Processing [Internet]. Wiley; 2011. (Food science and technology). Available from: https://books.google.pt/books?id=-ioIGmVPvZwC
14. Mogol BA, Gökmen V. Thermal process contaminants: acrylamide, chloropropanols and furan. Curr Opin Food Sci [Internet]. 2016;7:86–92. Available from: https://www.sciencedirect.com/science/article/pii/S2214799316000072
15. Mottram DS, Low MY, Elmore JS. The Maillard reaction and its role in the formation of acrylamide and other potentially hazardous compounds in foods. In: Skog K, Alexander J, editors. Acrylamide and Other Hazardous Compounds in Heat-Treated Foods [Internet]. Cambridge: Woodhead Publishing; 2006. p. 3–22. (Woodhead Publishing Series in Food Science, Technology and Nutrition). Available from: https://www.sciencedirect.com/science/article/pii/B978184569011350001X
16. Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J Agric Food Chem. 2002 Aug;50(17):4998–5006.

17. Murkovic M, Pedreschi F. Potentially Toxic Food Components Formed by Excessive Heat Processing. In: Uribarri J, editor. Dietary AGEs and Their Role in Health and Disease. 1st ed. Boca Raton, Florida: CRC Press; 2017. p. 87–101.
18. World Health Organization. Health Implications of Acrylamide in Food. World Health Organization. Geneva, Switzerland; 2002.
19. Zyzak D V, Sanders RA, Stojanovic M, Tallmadge DH, Eberhart BL, Ewald DK, et al. Acrylamide formation mechanism in heated foods. J Agric Food Chem. 2003 Jul;51(16):4782–7.
20. EFSA CONTAM Panel. Scientific Opinion on acrylamide in food. EFSA Journal. 2015;13(6).
21. Costa SA, Correia D, Carvalho C, Vilela S, Severo M, Lopes C, et al. Risk characterization of dietary acrylamide exposure and associated factors in the Portuguese population. Food Additives & Contaminants: Part A [Internet]. 2022 Mar 14;1–13. Available from: https://doi.org/10.1080/19440049.2022.2047540
22. Freisling H, Moskal A, Ferrari P, Nicolas G, Knaze V, Clavel-Chapelon F, et al. Dietary acrylamide intake of adults in the European Prospective Investigation into Cancer and Nutrition differs greatly according to geographical region. Eur J Nutr. 2013;52(4):1369–80.
23. Buyukdere Y, Akyol A. From a toxin to an obesogen: a review of potential obesogenic roles of acrylamide with a mechanistic approach. Nutr Rev [Internet]. 2024;82(1):128–42. Available from: https://www.scopus.com/inward/record.uri?eid=2-s2.0-85179836483&doi=10.1093%2Fnutrit%2Fnuad041&partnerID=40&md5=e41c905c04e38cf7a83d99326975b62c
24. Poteser M, Laguzzi F, Schettgen T, Vogel N, Weber T, Zimmermann P, et al. Time Trends of Acrylamide Exposure in Europe: Combined Analysis of Published Reports and Current HBM4EU Studies. Toxics. 2022 Aug;10(8).
25. Turkington C, Mitchell D. The encyclopedia of poisons and atidots. Third Edit. New York: Estate of Carol Turkington; 2009.
26. Miller MJ, Carter DE, Sipes IG. Pharmacokinetics of acrylamide in Fisher-344 rats. Toxicol Appl Pharmacol. 1982 Mar;63(1):36–44.
27. Sumner SC, Fennell TR, Moore TA, Chanas B, Gonzalez F, Ghanayem BI. Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. Chem Res Toxicol. 1999 Nov;12(11):1110–6.

28. Rifai L, Saleh FA. A Review on Acrylamide in Food: Occurrence, Toxicity, and Mitigation Strategies. Int J Toxicol. 2020;39(2):93–102.
29. Sarion C, Codină GG, Dabija A. Acrylamide in Bakery Products: A Review on Health Risks, Legal Regulations and Strategies to Reduce Its Formation. Vol. 18, International Journal of Environmental Research and Public Health. 2021.
30. Govindaraju I, Sana M, Chakraborty I, Rahman MH, Biswas R, Mazumder N. Dietary Acrylamide: A Detailed Review on Formation, Detection, Mitigation, and Its Health Impacts. Foods. 2024 Feb;13(4).
31. Hamdy SM, Bakeer HM, Eskander EF, Sayed ON. Effect of acrylamide on some hormones and endocrine tissues in male rats. Hum Exp Toxicol [Internet]. 2011 Aug 30;31(5):483–91. Available from: https://doi.org/10.1177/0960327111417267
32. Sto M, Matavulj M, Markovi J. Subchronic exposure to acrylamide leads to pancreatic islet remodeling determined by alpha cell expansion and beta cell mass reduction in adult rats. Acta Histochem. 2018;120(February):228–35.
33. Amato AA, Wheeler HB, Blumberg B. Obesity and endocrine-disrupting chemicals. Endocr Connect. 2021 Feb;10(2):R87–105.
34. De Long NE, Holloway AC. Early-life chemical exposures and risk of metabolic syndrome. Diabetes Metab Syndr Obes. 2017;10:101–9.
35. Wang B, Qiu W, Yang S, Cao L, Zhu C, Ma J, et al. Acrylamide exposure and oxidative dna damage, lipid peroxidation, and fasting plasma glucose alteration: Association and mediation analyses in chinese urban adults. Diabetes Care. 2020;43(7):1479–86.
36. Wan X, Zhu F, Zhuang P, Liu X, Zhang L, Jia W, et al. Associations of Hemoglobin Adducts of Acrylamide and Glycidamide with Prevalent Metabolic Syndrome in a Nationwide Population-Based Study. 2022;
37. Hung CC, Cheng YW, Chen WL, Fang WH. Negative Association between Acrylamide Exposure and Metabolic Syndrome Markers in Adult Population. Int J Environ Res Public Health. 2021 Nov;18(22).
38. Vesper HW, Slimani N, Hallmans G, Tjønneland A, Agudo A, Benetou V, et al. Cross-sectional study on acrylamide hemoglobin adducts in subpopulations from the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. J Agric Food Chem. 2008 Aug;56(15):6046–53.

39. Matoso V, Bargi-Souza P, Ivanski F, Romano MA, Romano RM. Acrylamide: A review about its toxic effects in the light of Developmental Origin of Health and Disease (DOHaD) concept. Food Chem [Internet]. 2019;283(January):422–30. Available from: https://doi.org/10.1016/j.foodchem.2019.01.054
40. Larsen PS, Kamper-Jørgensen M, Adamson A, Barros H, Bonde JP, Brescianini S, et al. Pregnancy and birth cohort resources in Europe: A large opportunity for aetiological child health research. Vol. 27, Paediatric and Perinatal Epidemiology. Blackwell Publishing Ltd; 2013. p. 393–414.
41. US Department of Agriculture Agricultural Research Service. USDA National Nutrient Database for Standard Reference: Nutrient Data Laboratory. www.ars.usda.gov. 2004.
42. Lopes C, Torres D, Oliveira A, Severo M, Guiomar S, Alarcão V, et al. National food, nutrition, and physical activity survey of the Portuguese general population (2015-2016): Protocol for design and development. J Med Internet Res [Internet]. 2018;20(2):1–11. Available from: www.ian-af.up.pt
43. Goios ACL, Severo M, Lloyd AJ, Magalhães VPL, Lopes C, Torres DPM. Validation of a new software eAT24 used to assess dietary intake in the adult Portuguese population. Public Health Nutr. 2020;(3):1–11.
44. Guerra RS, Fonseca I, Pichel F, Restivo MT, Amaral TF. Hand length as an alternative measurement of height. Eur J Clin Nutr. 2014;68(2):229–33.
45. WHO Multicentre Growth Reference Study Group. Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age Methods and development. Geneva; 2006.
46. Gibson RS. Principles of Nutritional Assessment. 2nd ed. New York: Oxford University Press; 2005.
47. Harrington DM, Staiano AE, Broyles ST, Gupta AK, Katzmarzyk PT. Waist circumference measurement site does not affect relationships with visceral adiposity and cardiometabolic risk factors in children. Pediatr Obes [Internet]. 2013 Jun 1;8(3):199–206. Available from: https://doi.org/10.1111/j.2047-6310.2012.00106.x
48. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Manuel Gómez J, et al. Bioelectrical impedance analysis - part II: utilization in clinical practice. Clinical Nutrition [Internet]. 2004 Dec 1;23(6):1430–53. Available from: https://doi.org/10.1016/j.clnu.2004.09.012

49. Schaefer F, Georgi M, Zieger A, Schärer K. Usefulness of bioelectric impedance and skinfold measurements in predicting fat-free mass derived from total body potassium in children. Pediatr Res. 1994 May;35(5):617–24.
50. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics. 2004 Aug;114(2 Suppl 4th Report):555–76.
51. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9.
52. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972 Jun;18(6):499–502.
53. World Medical Association. WMA Declaration of Helsinki: ethical principles for medical research involving human subjects. 1974;353(1):1418–9. Available from: http://www.wma.net/en/30publications/10policies/b3/index.html
54. Huang M, Zhuang P, Jiao J, Wang J, Zhang Y. Association of acrylamide hemoglobin biomarkers with obesity, abdominal obesity and overweight in general US population: NHANES 2003 – 2006. Science of the Total Environment [Internet]. 2018;631–632:589–96. Available from: https://doi.org/10.1016/j.scitotenv.2018.02.338
55. Chu PL, Lin LY, Chen PC, Su TC, Lin CY. Negative association between acrylamide exposure and body composition in adults: NHANES, 2003-2004. Nutr Diabetes. 2017 Mar;7(3):e246.
56. Liu Z, Wang J, Chen S, Xu C, Zhang Y. Associations of acrylamide with non-alcoholic fatty liver disease in American adults: a nationwide cross-sectional study. Environ Health. 2021 Aug;20(1):98.
57. Lin CY, Lin YC, Kuo HK, Hwang JJ, Lin JL, Chen PC, et al. Association among acrylamide, blood insulin, and insulin resistance in adults. Diabetes Care. 2009 Dec;32(12):2206–11.
58. Cheang I, Liao S, Zhu X, Lu X, Zhu Q, Yao W, et al. Association of acrylamide hemoglobin biomarkers with serum lipid levels in general US population: NHANES 2013-2016. Ecotoxicol Environ Saf. 2021 May;214:112111.

59. Yin G, Liao S, Gong D, Qiu H. Association of acrylamide and glycidamide haemoglobin adduct levels with diabetes mellitus in the general population. Environmental Pollution [Internet]. 2021;277:116816. Available from: https://www.sciencedirect.com/science/article/pii/S0269749121003961
60. Timmermann CAG, Mølck SS, Kadawathagedara M, Bjerregaard AA, Törnqvist M, Brantsæter AL, et al. A Review of Dietary Intake of Acrylamide in Humans. Toxics. 2021 Jun;9(7).
61. Huang M, Jiao J, Wang J, Chen X, Zhang Y. Associations of hemoglobin biomarker levels of acrylamide and all-cause and cardiovascular disease mortality among U.S. adults: National Health and Nutrition Examination Survey 2003–2006. Environmental Pollution [Internet]. 2018;238:852–8. Available from: https://www.sciencedirect.com/science/article/pii/S026974911733316X
62. Food Safety Authority of Ireland. Report on a total diet study carried out by the Food Safety Authority of Ireland in the period 2012 – 2014 [Internet]. 2016. Available from: www.fsai.ie
63. Zajac J, Bojar I, Helbin J, Kolarzyk E, Potocki A, Strzemecka J, et al. Dietary acrylamide exposure in chosen population of South Poland. Annals of Agricultural and Environmental Medicine. 2013;20(2):351–5.
64. European Commission. Commission Regulation (EU) 2017/2158: establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. Official Journal of the European Union [Internet]. 2017;L 304(315):24–44. Available from: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R2158&from=EN

Figures

Figure 1: Flowchart of the included participants with the selection criteria information.

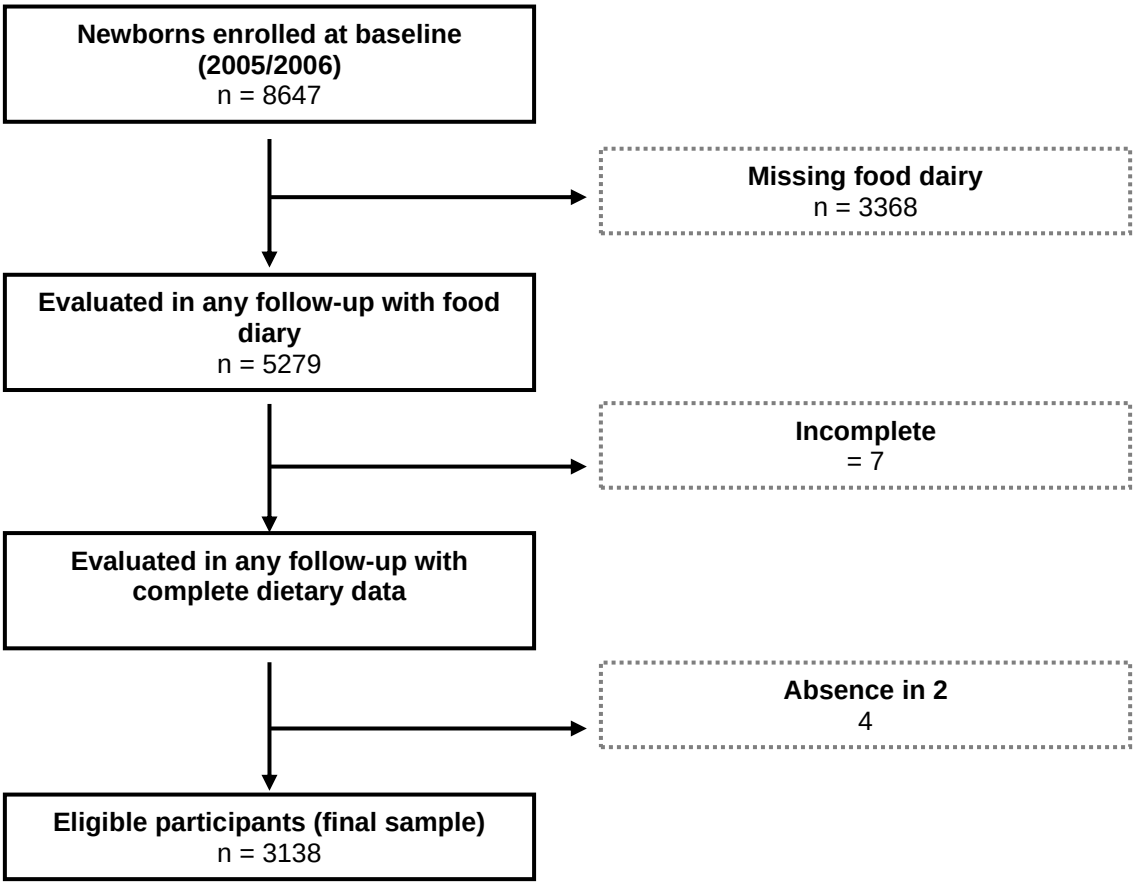
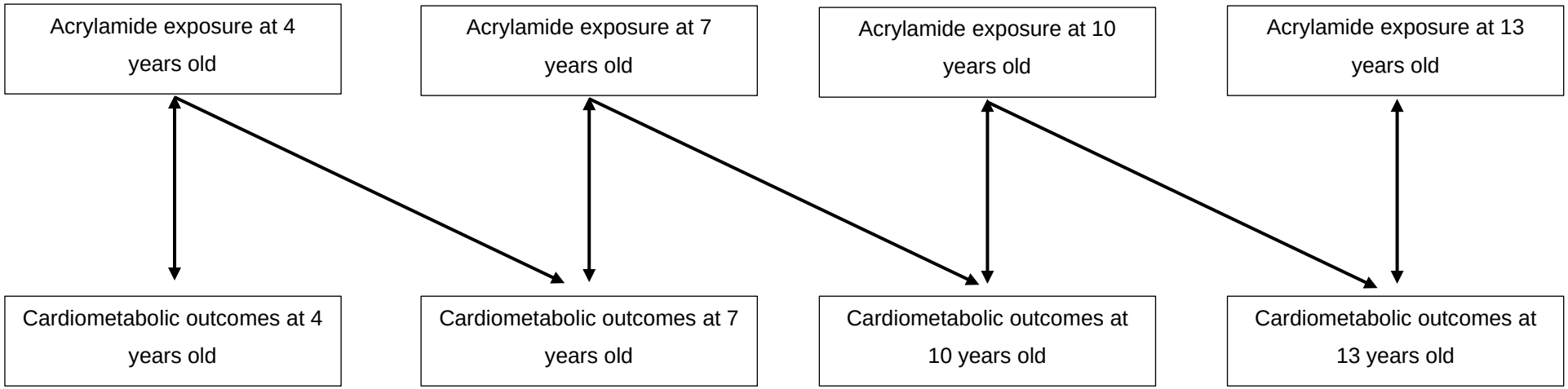


Figure 2: Theoretical framework of the associations between acrylamide daily dietary exposure and cardiometabolic outcomes (z-BMI, WC, %FM, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, blood glucose, blood insulin, HOMA-IR, z-SBP, z-DBP).



Abbreviations: z-BMI – body mass index z-score; WC – waist circumference; %FM – percentage of fat mass; LDL – low-density lipoprotein; HDL – high-density lipoprotein; HOMA-IR – homeostatic model assessment for insulin resistance; z-SBP – systolic blood pressure z-score; z-DBP – diastolic blood pressure z-score.

Tables

Table 1: Sociodemographic characteristics at baseline of the eligible participants and those of the remaining cohort.

	n	Eligible participants	Remaining cohort	p-value
Participant's sex*, n (%)*	8647			0.09
Female		1494 (47.8)	2743 (49.7)	
Male		1633 (52.2)	2777 (50.3)	
Maternal age (years)*, mean (SD)*	8636	30.8 (4.9)	28.8 (5.8)	**<0.001**
Maternal education (years)*, mean (SD)*	8591	11.6 (4.3)	9.8 (4.1)	**<0.001**

Abbreviations: n – sample size, % – percentage; SD – standard deviation.

Significant values are in **bold**.

Table 2: Leisure physical activity, cardiometabolic biomarkers and energy intake of eligible and non-eligible participants at 4-, 7-, 10- and 13-year follow-up.

	4y				7y				10y				13y			
	n	Eligible	Non-eligible	p-value	n	Eligible	Non-eligible	p-value	n	Eligible	Non-eligible	p-value	n	Eligible	Non-eligible	p-value
Leisure physical activity practice, *n (%)*	7383			0.25	4941			**<0.001**	6389			**<0.001**	4565			**<0.001**
Yes		2158 (70.3)	2981 (69.1)			1566 (60.2)	1164 (49.8)			2035 (66.7)	1882 (56.4)			1628 (61.7)	1040 (54.0)	
No		910 (29.7)	1334 (30.9)			1037 (39.8)	1174 (50.2)			1015 (33.3)	1456 (43.6)			1010 (38.3)	887 (46.0)	
z-BMI, *mean (SD)*	5934	0.56 (1.07)	0.66 (1.14)	**<0.001**	5833	0.70 (1.17)	0.73 (1.23)	0.26	5368	0.69 (1.22)	0.71 (1.26)	0.49	4637	0.41 (1.17)	0.51 (1.21)	**0.005**
Waist circumference (cm), *mean (SD)*	5919	52.5 (4.2)	53.0 (4.8)	**<0.001**	5825	58.9 (6.7)	59.4 (7.2)	**0.005**	5358	67.4 (9.5)	68.0 (10.1)	**0.03**	4627	73.8 (9.9)	74.9 (10.7)	**<0.001**
Percentage of fat mass (%), *mean (SD)*	4956	16.6 (7.7)	16.8 (8.2)	0.32	5440	17.2 (9.5)	17.7 (10.0)	0.05	5040	21.3 (10.8)	21.6 (11.2)	0.25	4511	25.1 (11.0)	26.0 (11.4)	**0.005**
Total cholesterol	1529	167.0 (26.9)	167.2 (28.4)	0.88	4571	168.9 (27.7)	169.4 (28.4)	0.52	3853	163.0 (26.9)	163.1 (27.4)	0.97	3779	147.9 (26.2)	147.6 (26.1)	0.80

(mg/dL), *mean (SD)*																
LDL cholesterol (mg/dL), *mean (SD)*	460	101.4 (23.6)	98.2 (22.9)	0.14	4548	99.4 (23.4)	99.7 (24.4)	0.74	3825	94.4 (22.6)	94.3 (23.1)	0.93	3718	84.0 (20.9)	83.4 (20.9)	0.35
HDL cholesterol (mg/dL), *mean (SD)*	1529	49.7 (10.0)	50.0 (10.2)	0.56	4573	56.2 (10.6)	55.9 (10.8)	0.38	3852	55.7 (10.5)	55.2 (10.8)	0.17	3781	51.4 (9.7)	51.4 (10.0)	0.95
Triglycerides (mg/dL), *mean (SD)*	1529	68.7 (30.8)	69.0 (30.3)	0.83	4573	67.7 (32.2)	70.4 (39.6)	**0.01**	3853	66.4 (31.8)	68.9 (36.8)	**0.03**	3781	66.2 (29.2)	67.4 (31.6)	0.255
Blood glucose (mg/dL), *mean (SD)*	1529	78.3 (8.1)	79.6 (8.5)	**0.003**	4572	82.9 (7.0)	83.4 (8.3)	**0.02**	3854	87.1 (7.8)	87.3 (7.8)	0.31	3780	89.1 (8.9)	89.1 (9.5)	0.88
Blood insulin (µU/mL), *mean (SD)*	1525	4.62 (4.82)	5.53 (6.52)	**0.002**	4020	5.49 (4.02)	5.76 (4.52)	0.05	3843	9.17 (5.95)	9.86 (6.71)	**<0.001**	3736	12.8 (7.1)	13.3 (10.3)	0.07
HOMA-IR, *mean (SD)*	1525	0.93 (1.13)	1.13 (1.46)	**0.003**	4016	1.15 (0.92)	1.21 (1.05)	0.05	3843	2.00 (1.36)	2.16 (1.55)	**<0.001**	3734	2.83 (1.72)	2.98 (2.58)	0.06

z-SBP, *mean (SD)*	4738	0.36 (0.71)	0.43 (0.78)	**0.001**	5812	0.70 (0.83)	0.72 (0.82)	0.32	5361	0.59 (0.85)	0.67 (0.91)	**<0.001**	4628	0.21 (0.93)	0.30 (0.95)	**0.002**
z-DBP, *mean (SD)*	4738	0.41 (0.71)	0.45 (0.72)	0.07	5812	1.03 (0.69)	1.07 (0.67)	**0.04**	5361	0.66 (0.62)	0.72 (0.65)	**<0.001**	4628	0.51 (0.77)	0.52 (0.76)	0.61
Energy intake (kcal/day), *mean (SD)*	2493	1604 (292)	1598 (300)	0.60	3587	1772 (305)	1740 (317)	**0.008**	2855	1910 (384)	1850 (383)	**0.007**	2833	1847 (433)	1819 (436)	0.14

Abbreviations: 4y – 4-year follow-up; 7y – 7-year follow-up; 10y – 10-year follow-up; 13y – 13-year follow-up; n – sample size, % – percentage; SD – standard deviation; z-BMI – body mass index z-score; LDL – low-density lipoprotein; HDL – high-density lipoprotein; HOMA-IR – homeostatic model assessment for insulin resistance; z-SBP – systolic blood pressure z-score; z-DBP – diastolic blood pressure z-score.

Significant values are in **bold**.

Table 3: Distribution of daily dietary exposure to acrylamide (µg/kg of body weight/day) and margins of exposure values for peripheral neuropathy and neoplastic effects, stratified by follow-up evaluations.

		Mean	P5*	P25*	Median	P75*	P95*
4-year follow-up *(n = 2013)*							
Dietary exposure	µg/kg/day	0.90	0.24	0.53	0.79	1.17	1.92
MOE_{PN}	-	478	224	368	544	811	1792
MOE_{NE}	-	189	89	145	215	321	708
7-year follow-up *(n = 3153)*							
Dietary exposure	µg/kg/day	0.73	0.24	0.44	0.65	0.92	1.47
MOE_{PN}	-	589	295	467	662	977	1792
MOE_{NE}	-	233	116	185	262	386	708
10-year follow-up *(n = 2641)*							
Dietary exposure	µg/kg/day	0.69	0.21	0.40	0.60	0.88	1.44
MOE_{PN}	-	623	299	489	717	1075	2048
MOE_{NE}	-	246	118	193	283	425	810
13-year follow-up *(n = 2692)*							
Dietary exposure	µg/kg/day	0.49	0.14	0.28	0.44	0.63	1.04
MOE_{PN}	-	878	413	683	977	1536	3071
MOE_{NE}	-	347	163	270	386	607	1214

Abbreviations: MOE_{PN} – margins of acrylamide exposure for peripheral neuropathy; MOE_{NE} – margins of acrylamide exposure for neoplastic effects; P_{α} – α-percentile.

MOE_{PN} considering $BMDL_{10}$ = 430 µg/kg of body weight/day, and MOE_{NE} considering $BMDL_{10}$ = 170 µg/kg of body weight /day.

* P_{α} MOE = $BMDL_{10}$ / $P_{1 - \alpha \text{ exposure}}$.

Table 4: Generalized least squares model of acrylamide daily dietary exposure (µg/day) on cardiometabolic outcomes (standardised β and 95% confidence interval (CI)).

	Model 1 std β (95% CI)	Model 2 std β (95% CI)	Model 3 std β (95% CI)
z-BMI			
Observations	5657	4982	4982
Acrylamide	**-0.03 (-0.05, -0.02)**	**-0.04 (-0.06, -0.02)**	**-0.04 (-0.06, -0.02)**
Acrylamide lag-1	**-0.06 (-0.08, -0.04)**	**-0.07 (-0.08, -0.05)**	**-0.07 (-0.09, -0.04)**
R^2	0.006	0.039	0.039
Waist circumference (cm)			
Observations	5653	4980	4980
Acrylamide	**0.10 (0.08, 0.12)**	**0.09 (0.06, 0.11)**	**0.09 (0.06, 0.12)**
Acrylamide lag-1	**0.12 (0.10, 0.14)**	**0.12 (0.09, 0.14)**	**0.06 (0.03, 0.08)**
R^2	-0.025	0.026	0.033
Percentage of fat mass (%)			
Observations	5387	4745	4745
Acrylamide	0.02 (-0.01, 0.04)	0.01 (-0.01, 0.03)	**0.05 (0.02, 0.08)**
Acrylamide lag-1	**0.08 (0.06, 0.10)**	**0.08 (0.06, 0.11)**	**0.04 (0.01, 0.06)**
R^2	-0.008	0.066	0.084
Total cholesterol (mg/dL)			
Observations	4552	4015	4015
Acrylamide	**-0.08 (-0.11, -0.06)**	**-0.08 (-0.10, -0.05)**	**-0.07 (-0.10, -0.04)**
Acrylamide lag-1	**-0.13 (-0.15, -0.10)**	**-0.13 (-0.16, -0.10)**	**-0.07 (-0.11, -0.04)**
R^2	0.014	0.021	0.028
LDL cholesterol (mg/dL)			
Observations	4495	3974	3974
Acrylamide	**-0.09 (-0.11, -0.07)**	**-0.09 (-0.11, -0.06)**	**-0.07 (-0.11, -0.04)**
Acrylamide lag-1	**-0.12 (-0.14, -0.09)**	**-0.12 (-0.15, -01.0)**	**-0.08 (-0.11, -0.04)**
R^2	0.013	0.020	0.025
HDL cholesterol (mg/dL)			
Observations	4543	4016	4016
Acrylamide	**-0.03 (-0.05, -0.00)**	-0.03 (-0.05, 0.00)	**-0.05 (-0.08, -0.02)**
Acrylamide lag-1	**-0.09 (-0.11, -0.06)**	**-0.09 (-0.12, -0.07)**	**-0.06 (-0.09, -0.02)**
R^2	0.000	0.008	0.016
Triglycerides (mg/dL)			
Observations	4543	4016	4016
Acrylamide	0.00 (-0.03, 0.02)	0.00 (-0.03, 0.03)	0.03 (-0.01, 0.06)
Acrylamide lag-1	-0.01 (-0.04, 0.01)	-0.00 (-0.04, 0.03)	0.02 (-0.02, 0.05)

R^2	0.000	0.012	0.015
Blood glucose (mg/dL)			
Observations	4542	4015	4007
Acrylamide	**0.07 (0.05, 0.10)**	**0.06 (0.04, 0.09)**	**0.08 (0.05, 0.11)**
Acrylamide lag-1	**0.07 (0.04, 0.10)**	**0.07 (0.04, 0.09)**	**0.03 (0.00, 0.07)**
R^2	0.007	0.021	0.025
Blood insulin (µU/mL)			
Observations	4369	3860	3860
Acrylamide	**0.05 (0.02, 0.08)**	**0.05 (0.02, 0.08)**	**0.08 (0.05, 0.12)**
Acrylamide lag-1	**0.09 (0.06, 0.12)**	**0.10 (0.06, 0.13)**	0.01 (-0.02, 0.05)
R^2	-0.027	0.014	0.036
HOMA-IR			
Observations	4369	3860	3854
Acrylamide	**0.05 (0.03, 0.08)**	**0.05 (0.02, 0.08)**	**0.08 (0.05, 0.12)**
Acrylamide lag-1	**0.09 (0.06, 0.11)**	**0.09 (0.06, 0.13)**	0.02 (-0.02, 0.05)
R^2	-0.025	0.011	0.030
z-SBP			
Observations	5648	4976	4976
Acrylamide	-0.01 (-0.04, 0.01)	-0.01 (-0.04, 0.01)	-0.02 (-0.05, 0.01)
Acrylamide lag-1	**-0.08 (-0.11, -0.06)**	**-0.10 (-0.12, -0.07)**	**-0.07 (-0.10, -0.03)**
R^2	0.001	0.022	0.024
z-DBP			
Observations	5648	4976	4976
Acrylamide	**-0.06 (-0.09, -0.04)**	**-0.06 (-0.09, -0.04)**	**-0.05 (-0.09, -0.02)**
Acrylamide lag-1	**-0.08 (-0.10, -0.05)**	**-0.08 (-0.10, -0.05)**	**-0.03 (-0.06, 0.00)**
R^2	0.010	0.024	0.029

Note: “Acrylamide” refers to cross-sectional exposure and “Acrylamide lag-1” refers to longitudinal exposure.

Abbreviations: std β – standardised regression coefficient; 95% CI – 95% confidence interval; Acrylamide lag-1 – acrylamide daily dietary exposure estimated for the previous follow-up evaluations (longitudinal); R^2 – R-squared; z-BMI – body mass index z-score; LDL – low-density lipoprotein; HDL – high-density lipoprotein; HOMA-IR – homeostatic model assessment for insulin resistance; z-SBP – systolic blood pressure z-score; z-DBP – diastolic blood pressure z-score.

Model 1: adjusted for cross-sectional and longitudinal acrylamide daily exposure values.

Model 2: adjusted for model 1 plus participants’ sex, maternal age, maternal education, maternal smoking habits, and practice of regular physical activity (cross-sectional and longitudinal).

Model 3: adjusted for model 2 plus total energy intake (kcal) (cross-sectional and longitudinal).

Significant values are in **bold**.

Appendices

Table S1: Generalized least squares model of acrylamide daily dietary exposure (µg/day) on cardiometabolic outcomes (standardised β and 95% confidence interval (CI)).

	Model 4 std β (95% CI)	Model 5 std β (95% CI)
Waist circumference (cm)		
Observations	4964	4963
Acrylamide	**-0.27 (-0.29, -0.25)**	**-0.02 (-0.04, -0.00)**
Acrylamide lag-1	**-0.21 (-0.23, -0.19)**	**-0.03 (-0.04, -0.01)**
R^2	0.227	0.439
Percentage of fat mass (%)		
Observations	-	4729
Acrylamide	**-**	**-0.08 (-0.10, -0.05)**
Acrylamide lag-1	**-**	**-0.04 (-0.06, -0.02)**
R^2	-	0.229
Total cholesterol (mg/dL)		
Observations	4007	4007
Acrylamide	**0.10 (0.08, 0.13)**	0.00 (-0.03, 0.02)
Acrylamide lag-1	**0.07 (0.04, 0.09)**	-0.01 (-0.03, 0.02)
R^2	0.016	0.115
LDL cholesterol (mg/dL)		
Observations	3966	3966
Acrylamide	**0.08 (0.05, 0.10)**	-0.02 (-0.04, 0.00)
Acrylamide lag-1	**0.05 (0.03, 0.08)**	-0.01 (-0.04, 0.01)
R^2	0.007	0.086
HDL cholesterol (mg/dL)		
Observations	4008	4008
Acrylamide	**0.14 (0.11, 0.17)**	**0.03 (0.00, 0.05)**
Acrylamide lag-1	**0.07 (0.04, 0.09)**	-0.01 (-0.03, 0.02)
R^2	0.033	0.068
Triglycerides (mg/dL)		
Observations	4008	4008
Acrylamide	**-0.03 (-0.06, -0.00)**	0.00 (-0.03, 0.03)
Acrylamide lag-1	-0.02 (-0.05, 0.01)	-0.00 (-0.04, 0.03)
R^2	0.015	0.013
Blood glucose (mg/dL)		
Observations	4007	4007
Acrylamide	**-0.07 (-0.09, -0.04)**	**0.03 (0.00, 0.06)**

Acrylamide lag-1	**-0.07 (-0.10, -0.04)**	-0.02 (-0.05, 0.01)
R^2	0.026	0.095
Blood insulin (µU/mL)		
Observations	3854	3854
Acrylamide	**-0.20 (-0.22, -0.17)**	**-0.03 (-0.06, -0.01)**
Acrylamide lag-1	**-0.13 (-0.16, -0.11)**	**-0.04 (-0.07, -0.01)**
R^2	0.108	0.277
HOMA-IR		
Observations	3854	3854
Acrylamide	**-0.19 (-0.22, -0.16)**	**-0.03 (-0.06, -0.00)**
Acrylamide lag-1	**-0.13 (-0.16, -0.10)**	**-0.04 (-0.06, -0.01)**
R^2	0.095	0.262
z-SBP		
Observations	4960	-
Acrylamide	**0.03 (0.01, 0.06)**	-
Acrylamide lag-1	-0.01 (-0.04, 0.01)	-
R^2	0.016	-
z-DBP		
Observations	4960	-
Acrylamide	0.01 (-0.02, 0.03)	-
Acrylamide lag-1	0.02 (-0.01, 0.04)	-
R^2	0.013	-

Note: “Acrylamide” refers to cross-sectional exposure and “Acrylamide lag-1” refers to longitudinal exposure.

Abbreviations: std β – standardised regression coefficient; 95% CI – 95% confidence interval; Acrylamide lag-1 – acrylamide daily dietary exposure estimated for the previous follow-up evaluations (longitudinal); R^2 – R-squared; LDL – low-density lipoprotein; HDL – high-density lipoprotein; HOMA-IR – homeostatic model assessment for insulin resistance; z-SBP – systolic blood pressure z-score; z-DBP – diastolic blood pressure z-score.

Model 4: adjusted for cross-sectional and longitudinal acrylamide daily exposure values, participants’ sex, maternal age, maternal education, maternal smoking habits, practice of regular physical activity (cross-sectional and longitudinal) and participants’ weight (kg) (cross-sectional and longitudinal).

Model 5: adjusted for cross-sectional and longitudinal acrylamide daily exposure values, participants’ sex, maternal age, maternal education, maternal smoking habits, practice of regular physical activity (cross-sectional and longitudinal) and participants’ height (m) (cross-sectional and longitudinal).

Significant values are in **bold**.

Paper IV

Association between bisphenol A exposure and cardiometabolic outcomes: A longitudinal approach

Sofia Almeida Costa, Milton Severo, Carla Lopes, Duarte Torres

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Association between bisphenol A exposure and cardiometabolic outcomes: A longitudinal approach

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HIGHLIGHTS

- BPA daily exposure estimated using data from direct and indirect methods.
- Random forest predicted BPA exposure, using food consumption data and urinary BPA.
- BPA was associated with higher blood insulin levels and insulin resistance.
- BPA was associated with higher fat mass, waist circumference, and body mass index.
- New food policies and strategies are need to reduce BPA exposure.

GRAPHICAL ABSTRACT

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ABSTRACT

Increased cardiometabolic risk is associated with abnormalities in blood biomarkers profile and adiposity measurements. Some substances found in the food matrix and the environment, called endocrine-disrupting chemicals, may impair cardiometabolic health in the early and later stages of life. Bisphenol A (BPA) is a food contaminant that migrates from food contact materials and may act as an endocrine disruptor, negatively affecting human health. The present work aims to longitudinally assess the association between BPA exposure and cardiometabolic outcomes, considering data from Portuguese population-based birth cohort Generation XXI. Blood insulin (0.06stdβ; 95 %CI:0.03,0.09) and insulin resistance (0.05stdβ; 95 %CI:0.02,0.08) presented a significant longitudinal association with BPA daily exposure after adjustment for important variables and energy. The same findings were observed for fat mass (0.03stdβ; 95 %CI 0.01,0.06) and waist circumference (0.06stdβ; 95 %CI:0.04,0.08). For z-BMI, a significant cross-sectional (0.03stdβ; 95 %CI:0.01,0.04) and longitudinal (0.02stdβ; 95 %CI:0.00,0.04) association was found. This was the first study assessing the association between BPA exposure and health outcomes from childhood to adolescence. We found an association between BPA exposure and increased blood insulin level, insulin resistance, fat mass percentage, waist circumference and z-BMI. Our results point to the need to reduce exposure to BPA in the early stages of life.

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1. Introduction

Abnormalities in the profile of biomarkers and adiposity measurements, such as hypertension, high glucose levels, dyslipidaemia, and central adiposity, are usually associated with an increased cardiometabolic risk in both adults and children [1-3]. Moreover, it has been suggested that, when occurring in the early stages of life, these profile modifications may negatively impact individual future health [4, 5,3].

Exposure to some substances found in the food matrix or the environment called endocrine-disrupting chemicals, may impair health factors related to the development of obesity, diabetes, and cardiovascular diseases [6]. Phthalates, dichlorodiphenyltrichloroethane (DDT), organochlorine pesticides, polychlorinated biphenyls (PCBs), dioxins and furans are some examples of endocrine disruptors [6]. Another concerning endocrine disruptor is bisphenol A (BPA), which may disrupt the endocrine system by interfering with nuclear estrogen receptors [7].

BPA is extensively used in the production of food contact materials, namely polycarbonate plastics, applied to food containers, reusable beverage bottles, tableware, cookware and microwave ovenware, and epoxy resins, lining the food and beverage cans [8,9]. Toys, medical devices, thermal paper, printing inks, electronic products, and flame retardants are other examples of non-food-related products that may contain BPA [10-12].

Globally, diet is considered the most important source of exposure to BPA for the human population, with canned food and beverages the higher contributors [13,14], followed by items packaged in specific plastics [15,16]. In Portugal, 57 % of the food items consumed by the population in 2015–2016 were reported with an associated package material, comprising 69 % plastic and 3 % metal [17].

Food diaries and 24-hour recalls are examples of indirect methods, frequently used to assess dietary exposure to diverse food contaminants, such as BPA [18]. Nevertheless, to better estimate food contaminants exposure, direct methods, as urinary biomarkers analysis, can be jointly with the indirect approaches [19,20]. As an advantage, direct methods can overcome the limitations associated to indirect methods (misreporting, participation bias, and identification of all food contaminant sources), by providing a more precise estimate [21,20,22].

In 2023, the European Food Safety Authority (EFSA) re-evaluated the risks to public health related to the presence of BPA in foodstuffs. The Authority updated Tolerable Daily Intake (TDI) to 0.2 ng/kg of body weight /day, considering the most recent data on the association between BPA exposure and health outcomes [8].

Some experimental animal studies, in vitro and in vivo, suggested the interference of BPA with multiple endocrine pathways, leading to the development of metabolic diseases and to the deregulation of specific hormonal systems. However, the results in human epidemiological studies remain inconclusive [8]. Some previous studies, conducted in different regions of the globe, demonstratedan association between BPA exposure and obesity, type 2 diabetes, metabolic syndrome, dyslipidaemia, cardiovascular diseases or hypertension [23-28], although some other studies found no association or contradictory results [23,29-34]. Additionally, most of these worldwide studies are cross-sectional, which does not allow the establishment of a causal association, and are conducted using small sample sizes and a single spot urine sample, largely limiting the strength of the reported findings.

Considering the lack of prospective studies, especially from childhood to adolescence, and studies that include important variables related to food intake as confounders, this study tries to address some uncertainties raised in the past.

The work aims to longitudinally assess the association between BPA exposure and cardiometabolic outcomes in children and adolescents, from 4- to 13-years old, using direct and indirect methods.

2. Material and methods

2.1. Study design and participants

Data from the Portuguese population-based birth cohort Generation XXI (GXXI) was used in the present study. GXXI is an ongoing prospective population-based birth cohort and included participants born between April 2005 and August 2006 at the five public units providing obstetrical and neonatal care in the metropolitan area of Porto, Portugal [35]. After delivery, 8 495 mothers and 8 647 children accepted to enrol in cohort GXXI, being all re-evaluated in the 4-, 7-, 10- and 13-year follow-ups, with a participation rate of 86 %, 80 %, 76 %, and 54 %, respectively. In March 2020, due to the COVID-19 pandemic, a 13-year follow-up was interrupted, reflecting the low participation rate observed in this wave.

To perform a longitudinal approach, this study used data collected from participants during follow-up evaluations. These data include a large amount of detailed information was collected, namely data from sociodemographic characteristics, lifestyle factors, dietary intake, anthropometric measurements, blood pressure, and blood and urine sample analytes.

Participants who provided complete food diaries (with at least two reported days) and had the outcome variable measured in at least 2 consecutive follow-ups were considered eligible. The flowchart of the included participants with the selection criteria information is described in Fig. 1.

2.2. Sociodemographic characteristics, lifestyle factors and dietary intake

Sex, maternal age at baseline and maternal educational level (defined as the number of completed schooling years) were the sociodemographic characteristics collected from the participants using standardised questionaries at baseline and updated in each follow-up evaluation.

Regarding lifestyle factors, parents or the primary caregiver were asked about the usual practice of some scheduled and regular sports activity outside the school setting at 4-, 7- and 10-year follow-ups. Adolescents were similarly questioned during the 13-year follow-up assessment. Regular physical activity (yes/no) was considered an important variable in the present study.

For the dietary intake assessment, parents or primary caregivers were invited to complete a 3-day food diary (including 2 weekdays and 1 weekend day) at the 4-, 7-, and 10-year follow-ups, while adolescents were invited to do the same at the 13-year follow-up. They received both oral and written instructions from the research team before an organised face-to-face interview. The participants provided detailed information about each food and beverage consumed: consumption place and time, food preparation method, brand, quantity consumed, and packaging materials (only for 13-year follow-up). The food diaries were reviewed by trained researchers during the face-to-face interview and codified *a posteriori* by a team of trained nutritionists into the Food Processor SQL software [36] at 4-, 7- and 10-year follow-ups and through "eAT24" software at 13-years follow-up. "eAT24" software was developed in the aim of the National Food, Nutrition and Physical Activity Survey (IAN-AF 2015–2016), which integrated the Portuguese Food Composition Table and adopted the FoodEx2 classification system, as described previously [37-39]. For the longitudinal approach, dietary information from 4-, 7- and 10-year follow-ups were subsequently harmonised in the "eAT24" software. Participants who reported at least two completed days in the food diary for each follow-up evaluation were considered eligible and included in the study analysis.

2.3. Anthropometrics measurements, blood pressure and blood samples: cardiometabolic outcomes

Body weight and height were measured by trained nurses under

standard procedures, i.e. after 12 h of fasting and with participants in light clothing and barefoot position [40,41]. Using a wall stadiometer (SECA, Hamburg, Germany) and a digital scale (SECA, Columbia, USA), the height was measured to the nearest 0.1 centimetre and body weight to the nearest 0.1 kg, respectively.

The body mass index (BMI) was calculated as weight over the squared height and computed according to age and sex-specific BMI standard deviation scores (z-BMI) published by the World Health Organization [42].

Waist circumference (WC) was measured at the umbilicus level to the nearest 0.1 centimetre, with the participant in a standing position, relaxed abdomen, arms at the sides and feet together [40,43].

Tetrapolar bioelectric impedance analysis was performed using BIA 101 Anniversary® (Akern, Florence, Italy), according to standard procedures [44]. The percentage of fat mass (%FM) was derived from the Schaefer et al. equation [45].

At 4- and 7-year follow-ups, after a 10-minute rest, with an interval of at least 5 min, using an automatic upper arm blood pressure monitor (Omron®) placed over the brachial artery pulse, two measurements of systolic (SBP) and diastolic (DBP) blood pressure were taken, and their mean value calculated. If the difference between the SBP and DBP measurements was > 5 mmHg, a third measurement was taken and considered the mean of the two closest values. At 10- and 13-year follow-ups, 3 measurements of SBP and DBP were taken, and the mean of the two closest values was calculated. For SBP and DBP, age-, sex- and height-specific z-scores (z-SBP and z-DBP, respectively) were calculated, according to the American Academy of Pediatrics [46].

On the morning of the follow-up evaluation, after a 12-hour overnight fast, a blood sample was collected for all participants who had provided consent. Blood biomarkers laboratory analyses were performed at the Clinical Pathology Department of the Hospital of São João, Porto, Portugal. Glucose was measured using a UV enzymatic assay (hexokinase method), and insulin was measured using an electrochemiluminescence immunoassay. A Homeostatic Model Assessment for Insulin Resistance value (HOMA-IR) [47] was calculated as a marker of insulin resistance. Enzymatic colourimetric assays were used to measure triglycerides (TG), total cholesterol and high-density lipoprotein-cholesterol (HDL cholesterol). The low-density lipoprotein-cholesterol (LDL cholesterol) was calculated using the Friedewald equation [48].

2.4. Urinary BPA

A convenience subsample of adolescents (n = 240) was invited to collect a 24-hour (24-h) urine sample, as close as possible to the interview, upon 30 days after the interview. The inclusion criterion was having a valid food diary at 7-, 10- and 13-year follow-ups. The research team gave oral and written instructions to the adolescents and their parents/primary caregivers, and all necessary material, such as BPA-free containers, was made available. Dispersive liquid-liquid microextraction followed by gas chromatography coupled with mass spectrometry was used to measure the total BPA (unconjugated and conjugated) in urine samples [49]. The BPA limit of detection (LOD) and quantification (LOQ) were 0.03 ng/mL and 0.1 ng/mL, respectively [49].

Due to incomplete data on dietary intake and missing values on the urinary output volume, 24 participants were excluded from the analysis (n = 216).

2.5. Ethics approval and consent to participate

The protocol of the GXXI birth cohort was approved by the Ethics Committee of São João University Hospital and registered with the Portuguese Individual Data Protection Authority. Written informed consent was obtained from parents or primary caregivers for all waves and oral assent from children/adolescents. GXXI complied with the national legislation and the Ethical Principles for Medical Research expressed in the Declaration of Helsinki. According to the European General Data Protection Regulation, all documents with identification

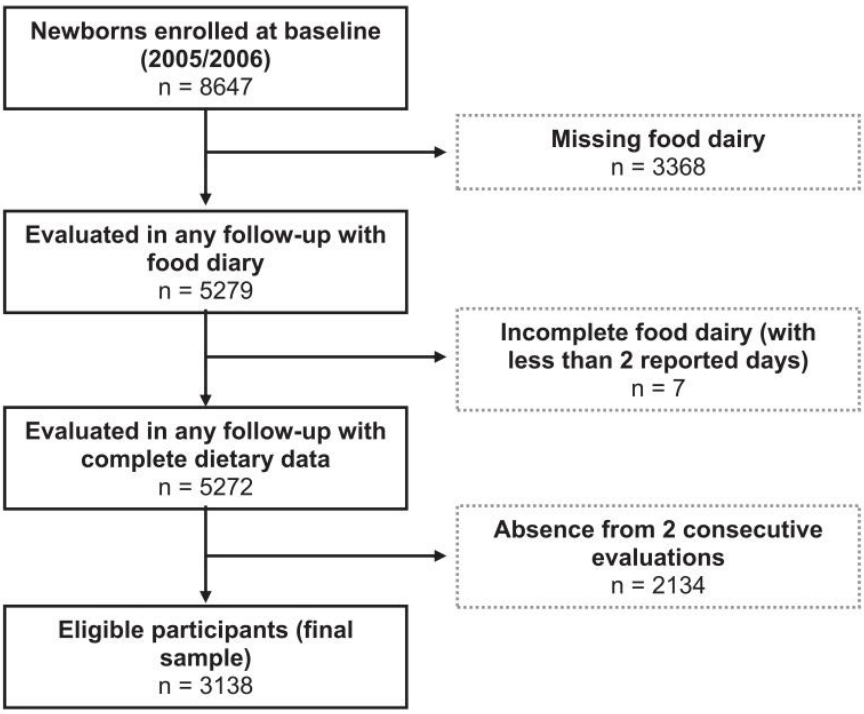

Fig. 1. Flowchart of the included participants with the selection criteria information.

data were treated separately and stored in a different dataset, databases were anonymised, and the Data Protection Officer ensured data protection at the Institute of Public Health of the University of Porto.

2.6. Statistical analysis

Since the packaging material was only collected in the 13-year follow-up, missing information on that wave and the previous waves were handled, attributing the proportions of the different packaging materials according each specific food item. This information was collected through the data on packaging materials reported by children and adolescents in IAN-AF 2015–2016, described elsewhere [50].

Total BPA daily excretion was calculated following the equation $E_{BPA} = C_{BPA} x\ V$, where E_{BPA} is the total BPA excreted in ng/day, C_{BPA} is the concentration of BPA measured in urine samples in ng/L, and V is the output urine volume in L/day. According to the right-skewed distribution observed for the total BPA measured in urine, a Box-Cox transformation was used, applying a calculated exponent of −0.1 for adolescents.

A random forest model was used to predict the total BPA daily exposure in the remaining sample and the remaining follow-up waves, considering data from the subsample that collected 24-h urine, as described previously [51]. The random forest combined information on food group consumption and food items reported with an associated packing material and the total BPA daily excretion measured from 24-h urine samples. A 2-times 5-fold cross-validation was applied to handle the possible overfitting of the model.

Associations between BPA daily exposure and cardiometabolic outcomes were tested using a generalised least squares model, according to the theoretical framework shown in Fig. 2, presented by standardised linear regression coefficient (std β) and 95 % confidence interval (95 % CI). Four separate models were applied: model 1 adjusted for cross-sectional and longitudinal BPA daily exposure predicted values; model 2 adjusted for model 1 plus participant sex, maternal age, maternal education, practice of regular physical activity (cross-sectional and longitudinal); model 3 adjusted for model 2 plus participant height (m) (cross-sectional and longitudinal); and model 4 adjusted for model 2 plus total energy intake (kcal) (cross-sectional and longitudinal). The correlation between participant height and total energy intake was tested using Pearson's correlation.

Sensitivity analyses were conducted to assess the impact of including or excluding participants diagnosed with diabetes, cardiovascular disease, and renal disease on the outcome.

For the remaining statistical analysis, missing data were treated at random. The R software version 4.2.1 for Windows was used, with a significance level of 5 %.

3. Results

From the initial cohort sample size (8647), 3368 were excluded, considering they did not provide any food diary during the different wave's evaluations, 7 due to incomplete data on dietary intake, and 2134 considering that they did not fulfil the criteria of being present in 2 consecutive follow-up evaluations. Thus, the final sample of this study was 3138 participants (Fig. 1). Sociodemographic characteristics at the baseline of eligible participants and those of the remaining cohort are present in Table 1. We found significant differences for all sociodemographic variables between eligible and non-eligible participants. Comparing with the remaining cohort, 52 % of the eligible participants were males, and their mothers had older maternal ages (30.8 ± 4.9 years vs 28.8 ± 5.8 years, $p < 0.001$) and higher levels of education (11.6 ± 4.3 years vs 9.8 ± 4.1 years, $p < 0.001$).

Table 2 presents the differences between characteristics of eligible and non-eligible participants at 4-, 7-, 10- and 13-year follow-ups. For most variables, no significant differences were found when comparing the participants in each follow-up evaluation. The 10-year follow-up was the evaluation with more significant differences found in characteristics between eligible and non-eligible participants. For the characteristics with significant differences, the eligible participants presented better health indicators than the non-eligible participants. The effect size ranged between −0.15 and 0.15 (data not shown). The mean daily exposure to BPA ranged from 1229 to 1295 ng/day at 4-year and 10-year follow-up evaluations, respectively, without significant differences, comparing eligible and non-eligible participants.

The cross-sectional and longitudinal associations between BPA daily exposure, estimated from 24-h urinary samples, and cardiometabolic outcomes are shown in Table 3. No associations were found for triglyceride serum levels. For the remaining serum lipids, blood glucose, z-SBP and z-DBP, we found a significant longitudinal association in models 1 and 2. However, after adjusting for participant height and

Table 1
Sociodemographic characteristics at baseline of the eligible participants and those of the remaining cohort.

	n	Eligible participants	Remaining cohort	p-value
Participant's sex, *n (%)*	8647			**0.07**
Female		1497 (47.7)	2740 (49.7)	
Male		1641 (52.3)	2769 (50.3)	
Maternal age (years), *mean (SD)*	8636	30.8 (4.9)	28.8 (5.8)	**<0.001**
Maternal education (years), *mean (SD)*	8591	11.6 (4.3)	9.8 (4.1)	**<0.001**

Abbreviations: n – sample size, % – percentage; SD – standard deviation.
Significant values are in **bold**.

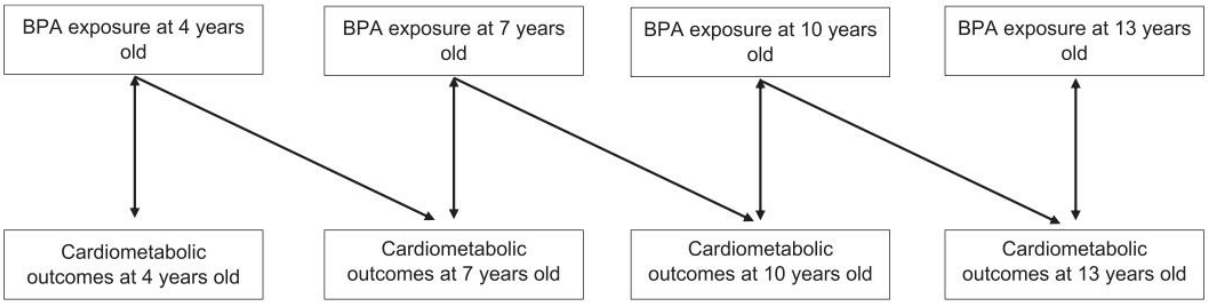

Fig. 2. Theoretical framework of the associations between BPA daily exposure and cardiometabolic outcomes (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, blood glucose, blood insulin, HOMA-IR, z-SBP, z- DBP, z-BMI, %FM, WC). Abbreviations: BPA – bisphenol A; LDL – low-density lipoprotein; HDL – high-density lipoprotein; HOMA-IR – homeostatic model assessment for insulin resistance; z-SBP – systolic blood pressure z-score; z-DBP – diastolic blood pressure z-score, z-BMI – body mass index z-score; %FM – percentage of fat mass; WC – waist circumference.

Table 2
Characteristics of eligible and non-eligible participants at 4-, 7-, 10- and 13-years follow-up.

	4 y				7 y				10 y				13 y			
	n	Eligible	Non-eligible	p-value	n	Eligible	Non-eligible	p-value	n	Eligible	Non-eligible	p-value	n	Eligible	Non-eligible	p-value
Physical activity practice, *n (%)*	7383			0.28	4941			**<0.001**	6389			**<0.001**	4565			**<0.001**
Yes		2164 (70.3)	2975 (69.1)			1567 (60.0)	1163 (49.9)			2040 (66.7)	1877 (56.3)			1630 (61.6)	1038 (54.0)	
No		915 (29.7)	1329 (30.9)			1043 (40.0)	1168 (50.1)			1018 (33.3)	1454 (43.7)			1014 (38.4)	883 (46.0)	
Total cholesterol (mg/dL), *mean (SD)*	1529	167.0 (27.0)	167.2 (28.3)	0.87	4571	168.9 (27.7)	169.5 (28.4)	0.49	3853	163.0 (26.9)	163.1 (27.4)	0.95	3779	147.9 (26.2)	147.6 (26.1)	0.81
LDL cholesterol (mg/dL), *mean (SD)*	460	101.4 (23.6)	98.2 (22.9)	0.14	4548	99.4 (23.4)	99.7 (24.4)	0.69	3825	94.4 (22.6)	94.4 (23.1)	0.95	3718	84.0 (20.9)	83.4 (20.9)	0.37
HDL cholesterol (mg/dL), *mean (SD)*	1529	49.7 (10.2)	49.9 (10.1)	0.80	4573	56.2 (10.6)	55.9 (10.8)	0.37	3852	55.7 (10.5)	55.2 (10.8)	0.17	3781	51.4 (9.7)	51.4 (10.0)	0.97
Triglycerides (mg/dL), *mean (SD)*	1529	68.5 (30.7)	69.1 (30.3)	0.69	4573	67.7 (32.2)	70.4 (39.6)	**0.01**	3853	66.4 (31.8)	68.9 (36.8)	**0.03**	3781	66.2 (29.2)	67.3 (31.7)	0.27
Blood glucose (mg/dL), *mean (SD)*	1529	78.3 (8.1)	79.6 (8.4)	**0.003**	4572	83.0 (7.6)	83.4 (7.6)	0.10	3854	87.1 (7.8)	87.3 (7.8)	0.32	3780	89.2 (9.4)	89.0 (8.8)	0.69
Blood insulin (μU/mL), *mean (SD)*	1525	4.61 (4.80)	5.54 (6.53)	**0.001**	4020	5.49 (4.04)	5.75 (4.51)	0.06	3843	9.16 (5.95)	9.86 (6.71)	**< 0.001**	3736	12.8 (7.1)	13.3 (10.3)	0.07
HOMA-IR, *mean (SD)*	1525	0.93 (1.13)	1.13 (1.47)	**0.002**	4016	1.15 (0.92)	1.20 (1.04)	0.08	3843	2.00 (1.36)	2.16 (1.55)	**< 0.001**	3734	2.84 (1.72)	2.98 (2.58)	0.06
z-SBP, *mean (SD)*	4738	0.36 (0.71)	0.43 (0.78)	**0.001**	5812	0.70 (0.83)	0.72 (0.82)	0.34	5361	0.59 (0.85)	0.66 (0.91)	**0.001**	4628	0.21 (0.93)	0.30 (0.95)	**0.003**
z-DBP, *mean (SD)*	4738	0.41 (0.71)	0.45 (0.72)	0.06	5812	1.03 (0.69)	1.07 (0.67)	**0.04**	5361	0.66 (0.62)	0.72 (0.65)	**< 0.001**	4628	0.51 (0.76)	0.52 (0.76)	0.60
z-BMI, *mean (SD)*	5934	0.56 (1.07)	0.66 (1.14)	**< 0.001**	5833	0.70 (1.17)	0.73 (1.23)	0.24	5368	0.69 (1.22)	0.71 (1.26)	0.45	4637	0.41 (1.17)	0.51 (1.21)	**0.006**
Percentage of fat mass (%), *mean (SD)*	4956	16.6 (7.7)	16.8 (8.2)	0.30	5440	17.2 (9.5)	17.7 (10.0)	**0.04**	5040	21.2 (10.8)	21.7 (11.2)	0.19	4511	25.1 (11.0)	26.0 (11.4)	**0.005**
Waist circumference (cm), *mean (SD)*	5919	52.5 (4.2)	53.0 (4.8)	**< 0.001**	5825	58.9 (6.7)	59.4 (7.2)	**0.005**	5358	67.4 (9.5)	68.0 (10.1)	**0.02**	4627	73.8 (9.9)	74.9 (10.7)	**< 0.001**
Energy intake (kcal/day), *mean (SD)*	2493	1604 (292)	1599 (301)	0.72	3587	1772 (305)	1740 (318)	**0.01**	2855	1910 (384)	1851 (384)	**0.008**	2833	1847 (433)	1820 (436)	0.16
BPA (ng/day), *mean (SD)*	2493	1229 (1540)	1229 (1523)	0.30	3587	1269 (1540)	1272 (1533)	0.78	2851	1295 (1548)	1300 (1558)	0.56	2804	1261 (1563)	1265 (1585)	0.56

Abbreviations: 4 y – 4-years follow-up; 7 y – 7-years follow-up; 10 y – 10-years follow-up; 13 y – 13-years follow-up; n – sample size, % – percentage; SD – standard deviation; LDL – low-density lipoprotein; HDL – high-density lipoprotein; HOMA-IR – homeostatic model assessment for insulin resistance; z-SBP – systolic blood pressure z-score; z-DBP – diastolic blood pressure z-score; z-BMI – body mass index z-score; BPA – bisphenol A. Significant values are in **bold**.

Table 3
Generalized least squares model of BPA daily exposure on cardiometabolic outcomes (standardised β and 95 % confidence interval (CI)).

	Model 1 std β (95 % CI)	Model 2 std β (95 % CI)	Model 3 std β (95 % CI)	Model 4 std β (95 % CI)
Total cholesterol (mg/dL)				
observations	4542	4067	4059	4067
BPA	-0.01 (−0.04, 0.01)	-0.01 (−0.03, 0.02)	-0.00 (−0.02, 0.02)	0.00 (−0.02, 0.03)
BPA lag-1	**-0.07 (−0.09, −0.04)**	**-0.06 (−0.08, −0.03)**	0.01 (−0.01, 0.04)	-0.02 (−0.04, 0.01)
R^2	0.003	0.010	0.115	0.027
LDL cholesterol (mg/dL)				
observations	4495	4026	4018	4026
BPA	-0.02 (−0.04, 0.01)	-0.01 (−0.04, 0.01)	-0.01 (−0.03, 0.02)	0.00 (−0.02, 0.03)
BPA lag-1	**-0.06 (−0.09, −0.04)**	**-0.05 (−0.08, −0.03)**	0.01 (−0.01, 0.03)	-0.02 (−0.04, 0.01)
R^2	0.002	0.007	0.085	0.023
HDL cholesterol (mg/dL)				
observations	4543	4068	4060	4068
BPA	-0.01 (−0.03, 0.02)	-0.01 (−0.03, 0.02)	-0.00 (−0.03, 0.02)	-0.01 (−0.04, 0.02)
BPA lag-1	**-0.03 (−0.06, −0.01)**	**-0.03 (−0.06, −0.01)**	0.02 (−0.00, 0.05)	-0.01 (−0.03, 0.02)
R^2	-0.001	0.006	0.066	0.014
Triglycerides (mg/dL)				
observations	4543	4068	4060	4068
BPA	0.01 (−0.02, 0.04)	0.01 (−0.02, 0.04)	0.01 (−0.02, 0.04)	0.02 (−0.01, 0.05)
BPA lag-1	-0.02 (−0.05, 0.01)	-0.01 (−0.04, 0.02)	-0.01 (−0.04, 0.02)	-0.00 (−0.03, 0.03)
R^2	0.000	0.011	0.013	0.014
Blood glucose (mg/dL)				
observations	4542	4067	4059	4067
BPA	0.01 (−0.01, 0.04)	0.01 (−0.02, 0.04)	0.01 (−0.02, 0.04)	0.00 (−0.03, 0.03)
BPA lag-1	**0.05 (0.03, 0.08)**	**0.05 (0.02, 0.08)**	0.00 (−0.02, 0.03)	0.03 (−0.00, 0.06)
R^2	0.003	0.017	0.088	0.020
Blood insulin (μU/mL)				
observations	4369	3912	3906	3912
BPA	-0.00 (−0.03, 0.02)	0.00 (−0.03, 0.03)	0.00 (−0.02, 0.03)	0.01 (−0.02, 0.04)
BPA lag-1	**0.09 (0.06, 0.12)**	**0.10 (0.07, 0.12)**	0.00 (−0.02, 0.03)	**0.06 (0.03, 0.09)**
R^2	-0.026	0.015	0.270	0.036
HOMA-IR				
observations	4369	3912	3906	3912
BPA	-0.00 (−0.03, 0.02)	-0.00 (−0.03, 0.03)	0.00 (−0.03, 0.03)	0.00 (−0.03, 0.03)
BPA lag-1	**0.09 (0.06, 0.11)**	**0.09 (0.06, 0.12)**	0.00 (−0.02, 0.03)	**0.05 (0.02, 0.08)**
R^2	-0.025	0.010	0.256	0.030
z-SBP				
observations	5648	5038	-	5038
BPA	0.01 (−0.02, 0.03)	0.01 (−0.02, 0.03)	-	0.01 (−0.02, 0.03)
BPA lag-1	**-0.05 (−0.07, −0.02)**	**-0.04 (−0.07, −0.02)**	-	-0.02 (−0.05, 0.01)
R^2	0.000	0.016	-	0.020
z-DBP				
observations	5648	5038	-	5038
BPA	-0.01 (−0.03, 0.02)	-0.00 (−0.03, 0.02)	-	0.01 (−0.02, 0.03)
BPA lag-1	**-0.05 (−0.08, −0.03)**	**-0.05 (−0.08, −0.02)**	-	-0.02 (−0.05, 0.01)
R^2	0.003	0.015	-	0.026
z-BMI				
observations	5657	5044	-	5044
BPA	**0.02 (0.00, 0.03)**	**0.02 (0.00, 0.03)**	-	**0.03 (0.01, 0.04)**
BPA lag-1	0.00 (−0.01, 0.02)	0.01 (−0.01, 0.02)	-	**0.02 (0.00, 0.04)**
R^2	0.000	0.019	-	0.025
Percentage of fat mass (%)				
observations	5387	4803	4787	4803
BPA	-0.00 (−0.02, 0.02)	-0.01 (−0.03, 0.02)	-0.01 (−0.04, 0.01)	0.00 (−0.02, 0.03)
BPA lag-1	**0.06 (0.03, 0.08)**	**0.06 (0.03, 0.08)**	-0.01 (−0.03, 0.01)	**0.03 (0.01, 0.06)**
R^2	-0.004	0.064	0.209	0.080
Waist circumference (cm)				
observations	5653	5042	5025	5042
BPA	**0.03 (0.01, 0.05)**	**0.02 (0.00, 0.04)**	0.00 (−0.01, 0.02)	0.01 (−0.01, 0.03)
BPA lag-1	**0.09 (0.07, 0.11)**	**0.09 (0.07, 0.11)**	**0.02 (0.01, 0.04)**	**0.06 (0.04, 0.08)**
R^2	-0.019	0.023	0.429	0.031

Abbreviations: std β – standardised regression coefficient; 95 % CI – 95 % confidence interval; BPA – bisphenol A (cross-sectional); BPA lag-1 – bisphenol A predicted for the previous follow-up evaluation (longitudinal); R2 – R-squared; LDL – low-density lipoprotein; HDL – high-density lipoprotein; HOMA-IR – homeostatic model assessment for insulin resistance; z-SBP – systolic blood pressure z-score; z-DBP – diastolic blood pressure z-score; z-BMI – body mass index z-score.
Model 1: adjusted for cross-sectional and longitudinal BPA daily exposure predicted values. Model 2: adjusted for model 1 plus participant sex, maternal age, maternal education, practice of regular physical activity (cross-sectional and longitudinal). Model 3: adjusted for model 2 plus participant height (m) (cross-sectional and longitudinal). Model 4: adjusted for model 2 plus total energy intake (kcal) (cross-sectional and longitudinal).
Significant values are in **bold**.

energy intake, the association did not remain statistically significant (models 3 and 4). Despite the absence of a significant association in model 3, which included height, blood insulin, and HOMA-IR, there was a significant longitudinal association with BPA daily exposure after adjustment for BPA predicted values, sociodemographic variables, practice of physical activity, participant height variables and energy (model 4). For each standard deviation increase in BPA exposure, there is a corresponding increase of 0.06 μU/mL (95 % CI: 0.03, 0.09) in blood

insulin and 0.05 (95 % CI: 0.02, 0.08) in HOMA-IR, respectively. The same findings were observed for %FM (model 4: 0.03 (95 % CI: 0.01, 0.06)). For z-BMI, a significant cross-sectional and longitudinal association was found in model 4 (cross-sectional: 0.03 (95 % CI: 0.01, 0.04); longitudinal: 0.02 (95 % CI: 0.00, 0.04)). A positive association was found between WC and BPA daily exposure, even after adjusting for all model variables. Each standard deviation increases in BPA exposure represented an increase of 0.06 cm (95 % CI: 0.04, 0.08) in WC (model 4).

The correlation between participant height and total energy intake in the present study was 0.25. According to the r-squared (Table 3), the proportion of variance explained by the urinary BPA concentration was low. Finally, sensitivity analyses, excluding participants diagnosed with diabetes, cardiovascular disease, and renal disease on the outcome, did not change the presented results.

4. Discussion

The present paper is one of the few prospective studies assessing the association between BPA daily exposure and cardiometabolic outcomes. A longitudinal association was found between BPA exposure and higher blood insulin levels, insulin resistance, fat mass percentage, waist circumference, and z-BMI. We found no evidence of an association between BPA and other metabolic outcomes, namely serum lipids, blood glucose, z-SBP and z-DBP. These findings remained the same when adjusted for important confounders, such as sex, maternal age, maternal education, physical activity, and total energy intake. However, only the associations of BPA exposure with waist circumference and z-BMI remain significant after adjustment for participants' height. Knowing that, in our study, higher height was correlated with higher energy consumption, we considered model 4 our final model. This study showed that early BPA exposure may impair cardiometabolic outcomes in future stages of life.

Considering the limited number of studies with longitudinal designs in younger ages, to the best of our knowledge, this is the first study that assessed the association of BPA exposure and health outcomes from childhood to adolescence, including a large variety of cardiometabolic biomarkers. Moreover, using data from food consumption and data of 24-h urinary excretion from a subsample at a 13-year follow-up, it was possible to estimate and predict BPA exposure for the remaining sample and all follow-up evaluations.

The originality of our study makes it difficult to compare with other studies, considering the differences in protocol design, sample size, year of sampling, type of urinary samples (24-hour urinary sample instead of a single spot), analytical method, approaches for urine dilution correction, and exposure assessment methodology. Comparing with the sample size of the present study, four cohort studies with a relatively small sample sizes in Greece (n = 235 and n = 500), the USA (n = 297), Japan (n = 396), and South Korea (n = 80) failed to find a longitudinal association between exposure to BPA during childhood and cardiometabolic endpoints [52,31,53,54]. In agreement with the Greece birth cohort [54], our study did not find a significant association between early BPA exposure and later profile modifications on total cholesterol, HDL cholesterol, z-SBP and z-DBP. However, contrary to our results, this Greek study did not find an association with increased BMI or other adiposity measures, including WC [54]. In the South Korean longitudinal study, including only preadolescent girls and applying repeated measures of multivariate analysis, the researchers did not find a prospective association of BPA exposure with BMI, blood insulin, blood glucose and HOMA-IR [53]. Lastly, in the Japanese and American children's cohorts, increased BMI was not associated with BPA exposure in the early stages of life [31,52]. Additionally, in the American study increased WC was also not associated with BPA exposure [31,52].

In adults of two cohort studies from France and China, an increased risk of type 2 diabetes, fasting glucose and beta cell dysfunction were associated with BPA early exposure [26,27]. However, in adults and in agreement with our findings, a sub-cohort from the EPIC study did not find an association between BPA and an increased incidence of hypertension [34].

Most previous evidence comes from cross-sectional design studies, with limited studies conducted in children or adolescents. In line with our results, data from different evaluations of the NHANES study, representative of the American population older than 6 years, failed to find an association between BPA exposure and serum lipids [29]. Also, in Chinese school children, it was observed a positive association between BPA exposure and BMI after adjustment for sex and age [28]. However, other published works found contradictory results compared to the ones presented here. A cross-sectional study with American children and adolescents only found a positive association with BMI $\geq$ 95th percentile and WC-to-height ratio $\geq$ 0.5, but no significant association was found for other variables such as BMI < 95th percentile, WC, %FM, HOMA-IR, glucose, TC, HDL cholesterol, LDL cholesterol and TG [30]. Recently, in Iran cross-sectional study of individuals aged 6–18 years, a positive association was found for BMI, WC, but also for SBP, DBP and glucose. As per our findings, this study did not find an association between BPA exposure and serum lipids [23].

Most published works used urinary spot samples to assess the exposure to BPA. However, due to their short half-life, a large intra-individual variability of BPA concentration in urine should be considered. In fact, some authors advocated that one sample is not recommended since it did not demonstrate good reliability and cannot account for possible misclassification of the participants [55-57]. In the present work, 24-h urine samples were used to estimate the daily BPA exposure, combining these data with food consumption information, representing one of the novel aspects of our study.

Although the mechanisms of BPA on cardiometabolic outcomes are not entirely understood, estrogen receptor signalling seems to play an important role in these processes [58-61]. BPA has the ability to mimics the action of estrogenic hormones, modifying the normal function of the endocrine system. The interaction of BPA with nuclear receptors, calcium ion channels and epigenetic mechanisms can disrupt the cardiovascular system, contributing to the development of cardiac interstitial fibrosis, arrhythmia, oxidative stress and atherosclerosis [62].

In the last decade, temporal trend analysis observed a slight reduction in BPA exposure [31], possibly associated with the proactive actions of food industries to replace this contaminant in their food contact materials, in addition to the established food policies [63–65]. Nevertheless, the implication of using BPA analogues in human health remains to clarify, hypothesising that these components may share a similar mode of action following oral exposure [14,66].

4.1. Limitations and strengths

Some limitations must be considered. First, the better health characteristics of the eligible participants indicate a possible participation bias. However, considering the sample size and the effect size, the results observed may be debatable from a clinical relevance perspective. Second, the underreporting of food packaging material collected by adolescents at 13-year follow-up evaluation. This situation was overcome through the available data from the National Food, Nutrition and Physical Activity Survey 2015–2016. However, due to the temporal line of the two studies, we must consider possible changes in the type of packaging material used by the food industry between 2005 and 2020. Lastly, the single effect of BPA on multifactorial outcomes, such as cardiometabolic, may be reductionist since consumers are exposed to a large variety of food matrix, nutrients, food contaminants and environmental endocrine disruptors.

This study also has several strengths. First, a prospective population-based birth cohort was used, with extensive collected information on sociodemographic characteristics, food consumption, blood biomarkers and urine samples, of a large sample. Secondly, the availability of 24-h urine samples allowed a more precise estimate of the total daily BPA

exposure. Third, data from direct and indirect methods were used to estimate the daily BPA exposure, an approach rarely considered in previous works. Moreover, by using a relatively limited number of urine samples, it was possible to estimate BPA daily exposure for the remaining sample and for the previous follow-up evaluations, which represents a significant strength. The longitudinal models were adjusted for several relevant confounders, including total energy intake, which is usually not considered in previous studies, representing another strength of this study. Finally, a highly sensitive method was used to determine the BPA urinary concentrations, with a low LOD (0.03 ng/mL) and LOQ (0.1 ng/mL), enabling an assessment of exposure to BPA at relatively low levels.

The present study answered some uncertainties raised in the past years regarding the assessment of exposure to BPA from childhood to later stages of life and their association with health outcomes. Thus, the need to globally reduce BPA daily exposure becomes urgent, since adverse health effects are expected when exposure chronically exceeds safety levels, even at low doses. The results of this study will help public health researchers all over the world, food safety agencies, and policy-makers to develop new and relevant guidelines, recommendations, and food policies.

5. Conclusion

This was the first study assessing the association between BPA exposure and health outcomes, including various biomarkers, from childhood to adolescence. Data from Portuguese population-based birth cohort Generation XXI was used. The present study showed that early BPA exposure can impair cardiometabolic outcomes in the future, namely higher blood insulin levels, insulin resistance, fat mass percentage, waist circumference and z-score of body mass index. The BPA daily exposure was estimated considering data from direct and indirect methods, rarely performed in previous works. We conclude that global strategies need to be developed and implemented to mitigate bisphenol A exposure. This demands modifications, not only in consumer behaviours, but also in the adoption of innovative approaches by the food and food packaging industries, to ensure a substantial reduction of BPA exposure.

Environmental implication

Exposure to some substances found in food matrix and in environment called endocrine disruptors, may impair health factors related to the development of obesity, diabetes, and cardiovascular diseases. One of these substances is bisphenol A (BPA), which may disrupt the endocrine system. BPA is extensively used in the production of food contact materials (polycarbonate plastics and epoxy resins), but also in non-food-related applications. There is a need to decrease BPA exposure through food packaging industry and by helping consumers to do better and healthy choices. This will also contribute to reduce the environmental impact of high consumption of packaged foods.

CRediT authorship contribution statement

Duarte Torres: Writing – review & editing, Supervision, Conceptualization. **Carla Lopes:** Writing – review & editing, Supervision. **Sofia Almeida Costa:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Milton Severo:** Writing – review & editing, Methodology, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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References

[1] Kim, M., Kim, J., 2022. Cardiometabolic risk factors and metabolic syndrome based on severity of obesity in Korean children and adolescents: data from the Korea National Health and Nutrition Examination Survey 2007–2018. Ann Pediatr Endocrinol Metab 27 (4), 289–299. https://doi.org/10.6065/apem.2142230.115.

[2] Lassale, C., Tzoulaki, I., Moons, K.G. M., & Sweeting, M. (2018). Europe PMC Funders Group Separate and combined associations of obesity and metabolic health with coronary heart disease: a pan-European case-cohort analysis. 39(5), 1–19. https://doi.org/10.1093/eurheartj/ehx448.Separate.

[3] Singleton, C.M.H., Brar, S., Robertson, N., DiTommaso, L., Fuchs, G.J., Schadler, A., et al., 2023. Cardiometabolic risk factors in South American children: a systematic review and meta-analysis. *PloS One* Vol. 18 (Issue 11). https://doi.org/10.1371/journal.pone.0293865.

[4] de Castro Silveira, J.F., Sehn, A.P., da Silva, L., Gaya, A.R., Lima, R.A., Burns, R.D., et al., 2023. The stability of cardiometabolic risk factors clustering in children and adolescents: a 2-year longitudinal study. J Diabetes Metab Disord 22 (1), 529–538. https://doi.org/10.1007/s40200-022-01174-1.

[5] Fonseca, M.J., Moreira, C., Santos, A.C., 2021. Adiposity rebound and cardiometabolic health in childhood: Results from the Generation XXI birth cohort. Int J Epidemiol 50 (4), 1260–1271. https://doi.org/10.1093/ije/dyab002.

[6] Kahn, L.G., Philippat, C., Nakayama, S.F., Slama, R., Trasande, L., 2020. Endocrine-disrupting chemicals: implications for human health. Lancet Diabetes Endocrinol 8 (8), 703–718. https://doi.org/10.1016/S2213-8587(20)30129-7.

[7] Giulivo, M., Lopez de Alda, M., Capri, E., Barceló, D., 2016. Human exposure to endocrine disrupting compounds: their role in reproductive systems, metabolic syndrome and breast cancer. A review. Environ Res 151, 251–264. https://doi.org/10.1016/j.envres.2016.07.011.

[8] European Food Safety Authority, 2023. Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA J 21 (4), e06857. https://doi.org/10.2903/j.efsa.2023.6857.

[9] Vandenberg, L.N., Hauser, R., Marcus, M., Olea, N., Welshons, W. v, 2007. Human exposure to bisphenol A (BPA). Reprod Toxicol 24 (2), 139–177. https://doi.org/10.1016/j.reprotox.2007.07.010.

[10] Kang, J.-H., Kondo, F., Katayama, Y., 2006. Human exposure to bisphenol A. Toxicology 226 (2), 79–89. https://doi.org/10.1016/j.tox.2006.06.009.

[11] Olea, N., Pulgar, R., Pérez, P., Olea-Serrano, F., Rivas, A., Novillo-Fertrell, A., et al., 1996. Estrogenicity of resin-based composites and sealants used in dentistry. Environ Health Perspect 104 (3), 298–305. https://doi.org/10.1289/ehp.96104298.

[12] Pulgar, R., Olea-Serrano, M.F., Novillo-Fertrell, A., Rivas, A., Pazos, P., Pedraza, V., et al., 2000. Determination of bisphenol A and related aromatic compounds released from bis-GMA-based composites and sealants by high performance liquid chromatography. Environ Health Perspect 108 (1), 21–27. https://doi.org/10.1289/ehp.0010821.

[13] Chang, W.H., Liu, S.C., Chen, H.L., Lee, C.C., 2019. Dietary intake of 4-nonylphenol and bisphenol A in Taiwanese population: Integrated risk assessment based on probabilistic and sensitive approach. Environ Pollut 244, 143–152. https://doi.org/10.1016/J.ENVPOL.2018.10.040.

[14] Liu, J., Wattar, N., Field, C.J., Dinu, I., Dewey, D., Martin, J.W., 2018. Exposure and dietary sources of bisphenol A (BPA) and BPA-alternatives among mothers in the APrON cohort study. Environ Int 119 (March)), 319–326. https://doi.org/10.1016/j.envint.2018.07.001.

[15] González, N., Marquès, M., Cunha, S.C., Fernandes, J.O., Domingo, J.L., Nadal, M., 2020. Biomonitoring of co-exposure to bisphenols by consumers of canned foodstuffs. Environ Int 140 (February), 105760. https://doi.org/10.1016/j.envint.2020.105760.

[16] Sirot, V., Rivière, G., Leconte, S., Leblanc, J.C., Kolf-Clauw, M., Vasseur, P., et al., 2021. Infant total diet study in France: exposure to substances migrating from food contact materials. Environ Int 149. https://doi.org/10.1016/j.envint.2021.106393.

[17] Costa, S.A., Vilela, S., Correia, D., Severo, M., Lopes, C., Torres, D., 2021. Consumption of packaged foods by the Portuguese population: type of materials and its associated factors. Br Food J 123 (2), 833–846. https://doi.org/10.1108/BFJ-07-2020-0584.

[18] de Fátima Poças, M., Hogg, T., 2007. Exposure assessment of chemicals from packaging materials in foods: a review. *Trends Food Sci Technol* Vol. 18 (Issue 4), 219–230. https://doi.org/10.1016/j.tifs.2006.12.008.

[19] Beckmann, M., Lloyd, A.J., Wilson, T., Torres, D.P.M., Goios, A.C.L., Willis, N.D., et al., 2020. Calystegines are potential urine biomarkers for dietary exposure to potato products. Mol Nutr Food Res 64 (20), 1–11. https://doi.org/10.1002/mnfr.202000515.

[20] Brouwer-Brolsma, E.M., Brennan, L., Drevon, C.A., Van Kranen, H., Manach, C., Dragsted, L.O., et al., 2017. Combining traditional dietary assessment methods with novel metabolomics techniques: Present efforts by the Food Biomarker Alliance. Proc Nutr Soc 76 (4), 619–627. https://doi.org/10.1017/S0029665117003949.

[21] Bingham, S.A., 2002. Biomarkers in nutritional epidemiology. Public Health Nutr 5 (6a), 821–827. https://doi.org/10.1079/PHN2002368.

[22] Gorecki, S., Bemrah, N., Roudot, A.-C., Marchioni, E., Le Bizec, B., Faivre, F., et al., 2017. Human health risks related to the consumption of foodstuffs of animal origin contaminated by bisphenol A. Food Chem Toxicol 110, 333–339. https://doi.org/10.1016/j.fct.2017.10.045.

[23] Amin, M.M., Ebrahim, K., Hashemi, M., Shoshtari-Yeganeh, B., Rafiei, N., Mansourian, M., et al., 2019. Association of exposure to Bisphenol A with obesity and cardiometabolic risk factors in children and adolescents. Int J Environ Health Res 29 (1), 94–106. https://doi.org/10.1080/09603123.2018.1515896.

[24] Delepierre, J., Fosse-Edorh, S., Fillol, C., Piffaretti, C., 2023. Relation of urinary bisphenol concentration and diabetes or prediabetes in French adults: a cross-sectional study. PLoS ONE 18 (3 March), 1–11. https://doi.org/10.1371/journal.pone.0283444.

[25] Moon, S., Hoon, S., Beom, C., Joo, Y., Joon, H., Sun, D., 2021. Effects of bisphenol A on cardiovascular disease: an epidemiological study using National Health and Nutrition Examination Survey 2003 – 2016 and meta-analysis. Sci Total Environ 763, 142941. https://doi.org/10.1016/j.scitotenv.2020.142941.

[26] Rancière, F., Botton, J., Slama, R., Lacroix, M.Z., Debrauwer, L., Charles, M.A., et al., 2019. Exposure to bisphenol a and bisphenol s and incident type 2 diabetes: a case-cohort study in the French cohort D.E.S.I.R. Environ Health Perspect 127 (10), 1–9. https://doi.org/10.1289/EHP5159.

[27] Wang, B., Li, M., Zhao, Z., Lu, J., Chen, Y., Xu, Y., et al., 2019. Urinary bisphenol A concentration and glucose homeostasis in non-diabetic adults: a repeated-measures, longitudinal study. Diabetologia 62 (9), 1591–1600. https://doi.org/10.1007/s00125-019-4898-x.

[28] Wang, H.X., Zhou, Y., Tang, C.X., Wu, J.G., Chen, Y., Jiang, Q.W., 2012. Association between bisphenol A exposure and body mass index in Chinese school children: A cross-sectional study. Environ Health: A Glob Access Sci Source 11 (1), 1–9. https://doi.org/10.1186/1476-069X-11-79.

[29] Dunder, L., Lejonklou, M.H., Lind, P.M., Lind, L., 2019. Urinary bisphenol A and serum lipids: a meta-analysis of six NHANES examination cycles (2003-2014). J Epidemiol Community Health 73 (11), 1012–1019. https://doi.org/10.1136/jech-2019-212555.

[30] Eng, D.S., Lee, J.M., Gebremariam, A., Meeker, J.D., Peterson, K., Padmanabhan, V., 2013. Bisphenol A and chronic disease risk factors in US children. Pediatrics 132 (3). https://doi.org/10.1542/peds.2013-0106.

[31] Gys, C., Ait, Y., Araki, A., Bastiaensen, M., Caballero-casero, N., Kishi, R., et al., 2020. Biomonitoring and temporal trends of bisphenols exposure in Japanese school children. Environ Res 191 (August)), 110172. https://doi.org/10.1016/j.envres.2020.110172.

[32] Lim, J., Choi, B.K., Jee, S.H., 2020. Urinary bisphenol A, phthalate metabolites, and obesity: do gender and menopausal status matter? Environ Sci Pollut Res 27 (27), 34300–34310. https://doi.org/10.1007/s11356-020-09570-x.

[33] Mouneimne, Y., Nasrallah, M., Khoueiry-Zgheib, N., Nasreddine, L., Nakhoul, N., Ismail, H., et al., 2017. Bisphenol A urinary level, its correlates, and association with cardiometabolic risks in Lebanese urban adults. Environ Monit Assess 189 (10). https://doi.org/10.1007/s10661-017-6216-8.

[34] Salamanca-Fernández, E., Vela-Soria, F., Rodríguez-Barranco, M., Arrebola-Moreno, A., Iribarne-Durán, L.M., Olea, N., et al., 2021. Serum levels of non-persistent environmental pollutants and risk of incident hypertension in a sub-cohort from the EPIC study, 193, 110491. https://doi.org/10.1016/j.envres.2020.110491.

[35] Larsen, P.S., Kamper-Jørgensen, M., Adamson, A., Barros, H., Bonde, J.P., Brescianini, S., et al., 2013. Pregnancy and birth cohort resources in Europe: a large opportunity for aetiological child health research (Blackwell Publishing Ltd). *Paediatr Perinat Epidemiol* Vol. 27 (Issue 4), 393–414. https://doi.org/10.1111/ppe.12060.

[36] US Department of Agriculture Agricultural Research Service. (2004). USDA National Nutrient Database for Standard Reference: Nutrient Data Laboratory. www.ars.usda.gov.

[37] European Food Safety Authority, 2011. Report on the development of a Food Classification and Description System for exposure assessment and guidance on its implementation and use. EFSA J 9 (12), 1–84. https://doi.org/10.2903/j.efsa.2011.2489.

[38] Goios, A.C.L., Severo, M., Lloyd, A.J., Magalhães, V.P.L., Lopes, C., Torres, D.P.M., 2020. Validation of a new software eAT24 used to assess dietary intake in the adult Portuguese population. Public Health Nutr 3, 1–11. https://doi.org/10.1017/S1368980020001044.

[39] Lopes, C., Torres, D., Oliveira, A., Severo, M., Guiomar, S., Alarcão, V., et al., 2018. National food, nutrition, and physical activity survey of the Portuguese general population (2015-2016): protocol for design and development. J Med Internet Res 20 (2), 1–11. https://doi.org/10.2196/resprot.8990.

[40] Gibson, R.S. (2005). Principles of Nutritional Assessment (2nd ed.). New York: Oxford University Press.

[41] Guerra, R.S., Fonseca, I., Pichel, F., Restivo, M.T., Amaral, T.F., 2014. Hand length as an alternative measurement of height. Eur J Clin Nutr 68 (2), 229–233. https://doi.org/10.1038/ejcn.2013.220.

[42] WHO Multicentre Growth Reference Study Group. (2006). Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age Methods and development.

[43] Harrington, D.M., Staiano, A.E., Broyles, S.T., Gupta, A.K., Katzmarzyk, P.T., 2013. Waist circumference measurement site does not affect relationships with visceral adiposity and cardiometabolic risk factors in children. Pediatr Obes 8 (3), 199–206. https://doi.org/10.1111/j.2047-6310.2012.00106.x.

[44] Kyle, U.G., Bosaeus, I., De Lorenzo, A.D., Deurenberg, P., Elia, M., Manuel Gómez, J., et al., 2004. Bioelectrical impedance analysis - part II: utilization in clinical practice. Clin Nutr 23 (6), 1430–1453. https://doi.org/10.1016/j.clnu.2004.09.012.

[45] Schaefer, F., Georgi, M., Zieger, A., Schärer, K., 1994. Usefulness of bioelectric impedance and skinfold measurements in predicting fat-free mass derived from total body potassium in children. Pediatr Res 35 (5), 617–624.

[46] National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents (2004). The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics, 114(2 Suppl 4th Report), 555–576.

[47] Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., Turner, R.C., 1985. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28 (7), 412–419. https://doi.org/10.1007/BF00280883.

[48] Friedewald, W.T., Levy, R.I., Fredrickson, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18 (6), 499–502.

[49] Cunha, S.C., Fernandes, J.O., 2010. Quantification of free and total bisphenol A and bisphenol B in human urine by dispersive liquid-liquid microextraction (DLLME) and heart-cutting multidimensional gas chromatography-mass spectrometry (MD-GC/MS). Talanta 83 (1), 117–125. https://doi.org/10.1016/j.talanta.2010.08.048.

[50] Lopes, C., Torres, D., Oliveira, A., Severo, M., Alarcão, V., Guiomar, S. et al. (2018). National Food, Nutrition, and Physical Activity Survey of the Portuguese General Population, IAN-AF 2015–2016: Summary of Results. www.ian-af.up.pt.

[51] Costa, S.A., Severo, M., Correia, D., Carvalho, C., Magalhães, V., Vilela, S., et al., 2023. Methodological approaches for the assessment of bisphenol A exposure. Food Res Int 173, 113251. https://doi.org/10.1016/j.foodres.2023.113251.

[52] Braun, J.M., Lanphear, B.P., Calafat, A.M., Deria, S., Khoury, J., Howe, C.J., et al., 2014. Early-life bisphenol a exposure and child body mass index: a prospective cohort study. Environ Health Perspect 122 (11), 1239–1245. https://doi.org/10.1289/ehp.1408258.

[53] Lee, H.A., Kim, Y.J., Lee, H., Gwak, H.S., Park, E.A., Cho, S.J., et al., 2013. Effect of urinary bisphenolA on androgenic hormones and insulin resistance in preadolescent girls: a pilot study from the Ewha Birth & Growth Cohort. Int J Environ Res Public Health 10 (11), 5737–5749. https://doi.org/10.3390/ijerph10115737.

[54] Vafeiadi, M., Roumeliotaki, T., Myridakis, A., Chalkiadaki, G., Fthenou, E., Dermitzaki, E., et al., 2016. Association of early life exposure to bisphenol A with obesity and cardiometabolic traits in childhood. Environ Res 146, 379–387. https://doi.org/10.1016/j.envres.2016.01.017.

[55] Gys, C., Bastiaensen, M., Bruckers, L., Colles, A., Govarts, E., Rodriguez, L., et al., 2021. Determinants of exposure levels of bisphenols in flemish adolescents. Environ Res 193 (December 2020)), 110567. https://doi.org/10.1016/j.envres.2020.110567.

[56] Husøy, T., Andreassen, M., Hjertholm, H., Carlsen, M.H., Norberg, N., Sprong, C., et al., 2019. The Norwegian biomonitoring study from the EU project EuroMix: levels of phenols and phthalates in 24-hour urine samples and exposure sources from food and personal care products. Environ Int 132 (March)). https://doi.org/10.1016/j.envint.2019.105103.

[57] Pollack, A.Z., Perkins, N.J., Sjaarda, L., Mumford, S.L., Kannan, K., Philippat, C., et al., 2016. Variability and exposure classification of urinary phenol and paraben

metabolite concentrations in reproductive-aged women. Environ Res 151, 513–520. https://doi.org/10.1016/j.envres.2016.08.016.
[58] Cimmino, I., Fiory, F., Perruolo, G., Miele, C., Beguinot, F., Formisano, P., et al., 2020. Potential mechanisms of bisphenol A (BPA) contributing to human disease. Int J Mol Sci 21 (16). https://doi.org/10.3390/ijms21165761.
[59] Delfosse, V., Grimaldi, M., Cavaillès, V., Balaguer, P., Bourguet, W., 2014. Structural and functional profiling of environmental ligands for estrogen receptors. Environ Health Perspect 122 (12), 1306–1313. https://doi.org/10.1289/ehp.1408453.
[60] Fenichel, P., Chevalier, N., Brucker-Davis, F., 2013. Bisphenol A: an endocrine and metabolic disruptor. Ann d'endocrinologie 74 (3), 211–220. https://doi.org/10.1016/j.ando.2013.04.002.
[61] Mustieles, V., D'Cruz, S.C., Couderq, S., Rodríguez-Carrillo, A., Fini, J.-B., Hofer, T., et al., 2020. Bisphenol A and its analogues: a comprehensive review to identify and prioritize effect biomarkers for human biomonitoring. Environ Int 144, 105811. https://doi.org/10.1016/j.envint.2020.105811.
[62] Zhang, Y.-F., Shan, C., Wang, Y., Qian, L.-L., Jia, D.-D., Zhang, Y.-F., et al., 2020. Cardiovascular toxicity and mechanism of bisphenol A and emerging risk of bisphenol S. Sci Total Environ 723, 137952. https://doi.org/10.1016/j.scitotenv.2020.137952.
[63] European Commission (2011a)., 2011. Commission Implementing Regulation (EU) No 321/2011 of 1 April 2011 amending Regulation (EU) No 10/2011 as regards the restriction of use of Bisphenol A in plastic infant feeding bottles. Off. J. Eur. Union L 87, 1–2. http://data.europa.eu/eli/reg_impl/2011/321/oj.
[64] European Commission, 2011. Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. In: Official Journal of the European Union, L, 12, pp. 1–89. ⟨https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:012:0001:0089:EN:PDF⟩.
[65] European Commission, 2018. Commission Regulation (EU) No 2018/213 of 12 February 2018 on the use of bisphenol A in varnishes and coatings intended to come into contact with food and amending Regulation (EU) No 10/2011 as regards the use of that substance in plastic food contact mat. In: Official Journal of the European Union, L, 41, pp. 6–12. ⟨http://data.europa.eu/eli/reg/2018/213/oj⟩.
[66] Rochester, J.R., Bolden, A.L., 2015. Bisphenol S and F: a systematic review and comparison of the hormonal activity of bisphenol A substitutes. Environ Health Perspect 123 (7), 643. https://doi.org/10.1289/EHP.1408989.

Appendice

Table A1: Distribution of daily dietary exposure to bisphenol A (ng/kg of body weight/day) of the eligible participants, stratified by follow-up evaluations.

	P5	P25	Median	P75	P95
4-year follow-up	48.7	60.8	68.8	77.8	90.6
7-year follow-up	33.9	43.5	50.3	57.6	67.9
10-year follow-up	23.4	30.1	36.3	42.1	51.7
13-year follow-up	16.1	21.0	24.7	29.0	36.3

Abbreviations: P_{α} – α-percentile.

Paper V

Associations between food contaminants exposure and pubertal development at 10 and 13 years old: findings from Generation XXI cohort

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Submitted

Associations between food contaminants exposure and pubertal development at 10 and 13 years old: findings from Generation XXI cohort

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Highlights

- AA and BPA daily exposure was estimated through collected dietary information
- AA and BPA exposure may impair pubertal development at 10 and 13 years old
- AA exposure was negatively associated with pubertal development in girls
- BPA exposure was positively associated with pubertal development in boys
- The combined exposure effect did not significantly change the found associations

Abstract

Endocrine-disrupting compounds, such as acrylamide (AA) and bisphenol A (BPA), are external substances usually found in air, water, food and other consumer products that may influence puberty timing onset.

This study aims to assess the association of food contaminants exposure, namely AA and BPA, individually and combined, on pubertal development in children and adolescents aged 4 to 13.

Data from four waves of the Portuguese population-based birth cohort Generation XXI was used (n = 5279). Dietary information was gathered through 3-day food diaries and categorised using the FoodEx2 classification system. Daily dietary AA exposure was estimated by merging food consumption with occurrence data from EFSA's publication. Daily BPA exposure was estimated using a random forest model that integrated food consumption data with urinary BPA levels. Linear regression models were used to test the associations between exposure to food contaminants (individually and combined), and pubertal development. Two models were performed: Model 1 adjusted for the exact participants' age, maternal age, maternal education, and the practice of leisure physical activity, and Model 2 adjusted for Model 1 plus total energy intake.

The median dietary exposure is higher in boys for both AA and BPA, decreasing with age in both sexes. After adjusting for all confounders (model 2), a significant negative association was found between individual AA exposure at 7 years (-0.007 (95% CI: -0.013, -0.002)), 10 years (-0.006 (95% CI: -0.010, -0.003)) and 13 years (-0.005 (95% CI: -0.009, -0.001)) and the pubertal development global score in girls, and a significant positive association for individual AA exposure at 13-years (0.003 (95% CI: 0.000, 0.007)) in boys. A significant positive association between BPA exposure at 10 and 13 years and a higher pubertal development global score was only found in boys. Testing the combined exposure did not significantly change the results observed for the individual exposure to each food contaminant, except for the AA association in boys that lost their significance.

Childhood and adolescent exposure to AA and BPA was associated with impaired timing of puberty onset, representing a relevant public health concern.

Keywords: cohort, acrylamide, bisphenol A, children, adolescents, Tanner

Introduction

Puberty represents a dynamic transition from childhood to adulthood, with significant physical changes leading to reproductive maturity. However, the timing of pubertal onset and the speed of its progression vary greatly among individuals and may be influenced by external factors (1).

In recent years, the development of different endocrine abnormalities due to exposure to endocrine-disrupting compounds (EDC) from the environment has been well described (2). EDC are external substances usually found in air, water, food, and other consumer products, and they are one of the factors that may influence puberty timing (3,4). These compounds may directly interfere with estrogenic or androgenic signalling pathways and consequently with the hypothalamic-pituitary-gonadal axis or indirectly with peripheral organs, such as adipose tissue and adrenal glands (5).

Considering that the correct function of the endocrine system is essential for the control, development and maturation of the different tissues and organs, EDC exposure in critical time windows can significantly impair short- and long-term health outcomes (4). Prenatal period, namely the embryo-fetal time, and postnatal period, childhood and adolescence, are the most susceptible to disruptions (1,4).

Acrylamide (AA) is a compound primarily formed via the Maillard reaction when the amino acid asparagine reacts with reducing sugar under low-moisture conditions at temperatures exceeding 120ºC. It is commonly found in baked or fried carbohydrate-rich foods, tobacco smoke, and other industrial applications (4,6,7). For the general population, particularly children and adolescents, diet represents the primary source of AA exposure (8). Major dietary contributors include cereal products (such as bread, toast, breakfast cereals, biscuits, and snacks), potato-based foods (including chips, French fries, and crisps), coffee and coffee substitutes (8,9). AA was classified as “probably carcinogenic” by the International Agency for Research on Cancer in 1994 (10). Moreover, recently, AA was also considered a potential EDC (4,11), corroborated by animal studies which found an association with reproductive toxicity, including ovarian dysfunction, disrupted spermatogenesis and infertility (4). However, epidemiological studies in humans, particularly testing the association between

AA exposure at younger ages and pubertal development, are limited and inconclusive.

Bisphenol A (BPA) is a monomer used to produce polycarbonate plastics and epoxy resins. Polycarbonate plastics are commonly applied to food contact materials, such as reusable beverage bottles, tableware, cookware, and food containers. Epoxy resins are often used in protective linings of food and beverage cans (12,13). Additionally, BPA is utilised in different non-food-related products, including toys, medical devices, thermal paper, electronic products, and flame retardants (14). Diet is considered the main source of BPA exposure in humans, with canned foods and beverages being the most relevant contributors (15,16). This food contaminant was considered EDC, and considering both the estrogenic and anti-androgenic effects after exposure may impair the reproductive system and pubertal regulation (17,18). Despite that, previous studies testing pre- and post-natal BPA exposure and consequences on pubertal development have shown controversial results, leaving the conclusions of this association unclear.

Different possible explanations for the inconclusive results found in the previously published studies regarding EDC and pubertal development may be related to the type of EDC, duration of exposure, type of outcomes and age at exposure/ outcome evaluation (19). Furthermore, most of the studies have been focused on assessing the effect of individual compounds, with a narrow perspective considering the complexity of the diet and the real-world scenario of food contaminants exposure.

Thus, this study aims to assess the association of food contaminants exposure, namely acrylamide and bisphenol A, individually and combined, on pubertal development in children and adolescents aged 4 to 13.

Methods

Study design and participants

This study utilised data from Generation XXI (GXXI), an ongoing prospective population-based birth cohort established between April 2005 and August 2006, across five public healthcare units providing obstetrical and neonatal care in the metropolitan area of Porto, Portugal, as previously described (20). Recruitment was based on the following eligibility criteria: mothers residing in one of the six municipalities within the metropolitan area of Porto, delivering at the public

hospitals serving these municipalities, and giving birth to live infants with a gestational age of at least 24 weeks. Between 24 to 72 hours post-delivery, mothers were invited to join the GXXI cohort, with a participation rate of 91%, resulting in the enrolment of 8,495 mothers and 8,647 children. Participants were subsequently invited for follow-up assessments at 4, 7, 10, and 13 years of age, with participation rates of 86%, 80%, 76%, and 54%, respectively. The lower participation rate of the last follow-up was attributed to the COVID-19 pandemic, which prematurely interrupted data collection in March 2020.

This study analysed data from all follow-up evaluations. The eligibility criteria for inclusion were having at least one completed food diary, i.e. at least two reported days within each follow-up, and having performed a sexual development evaluation at 10 and/or at 13 years old. Our final sample included 5279 participants.

Sociodemographic characteristics and lifestyle factors

Sociodemographic characteristics were collected from the participants and mothers using standardised questionaries. Maternal age at baseline and maternal educational level (defined as the number of completed schooling years) were the variables considered and collected at baseline. The child's sex was collected at baseline, and the exact age of the child was calculated based on the date on which the pubertal development assessment was conducted.

Regarding lifestyle factors, physical activity was considered a potential confounding variable in the association between exposure to food contaminants and pubertal development impairment. Thus, the practice (yes/no) of leisure physical activity outside the school was used at 4-, 7-, 10-, and 13-year follow-ups.

Dietary intake and exposure to food contaminants

Before the face-to-face interview, parents (or primary caregivers) at the 4-, 7-, and 10-year follow-ups, as well as adolescents at the 13-year follow-up, were instructed by the research team, both orally and in writing, to complete a 3-day food diary, including two weekdays and one weekend day. Detailed information was collected for each food or beverage consumed, such as the time and place of consumption, brand, preparation method, cooking process, and quantity

consumed. During the face-to-face interviews, trained researchers reviewed the food diaries. Trained nutritionists coded data from the 4-, 7-, and 10-year follow-ups using Food Processor SQL software (21) and processed data from the 13-year follow-up with the "eAT24" software. Dietary data from the 4- to 10-year follow-ups were harmonised in the "eAT24" software to ensure consistency. This software, developed for the Portuguese National Food, Nutrition, and Physical Activity Survey 2015-2016, integrates the Portuguese Food Composition Table and uses the FoodEx2 classification system, as previously described (22,23). Finally, all food items and beverages were converted into energy and nutrients, and the individual mean daily intake was computed. Participants were eligible for inclusion in the present study only if their food diaries included data for at least two complete days in any follow-up evaluation.

The daily dietary exposure to AA (µg/kg of body weight/day) was then calculated following previously described methods (9). Data on AA levels in various food groups were obtained from the EFSA Scientific Opinion on Acrylamide in Food, published in 2015 (8). Ten cycles of random assignments were conducted, producing ten distinct estimated AA values for each consumption occasion.

A random forest model was used to estimate the daily exposure to BPA, as described elsewhere (24). This methodology combined information on food group consumption, food packing material, and the total urinary concentration of BPA measured in 24-h urine of a sub-sample. A 2-times 5-fold cross-validation was applied to handle the possible overfitting of the model. The random forest was then applied to predict the total BPA daily exposure in the remaining sample and the remaining follow-up waves.

Anthropometrics measurements

Trained observers performed a physical examination and anthropometric measures at the 4-, 7-, 10- and 13-year-old follow-up evaluations. Height and weight were measured objectively during face-to-face interviews following standard procedures. Measurements were taken after a 12-hour fasting period, with participants dressed in light clothing and barefoot (25). Height was recorded to the nearest centimetre using a wall stadiometer (SECA, Hamburg, Germany), while body weight was measured to the nearest tenth of a kilogram using a digital scale (SECA, Columbia, USA). Body mass index (BMI) was calculated as weight

divided by height squared, and participants were classified based on age- and sex-specific BMI z-scores (z-BMI) provided by the World Health Organization (26).

Pubertal development

Pubertal development was assessed at ages 10 and 13 using the Tanner scale, a standardised method for evaluating sexual maturation (27,28). The scale encompasses different parameters, namely breast development in girls, genital development in boys and pubic hair development in both sexes. At 13 years old, axillary hair was evaluated for both sexes. The evaluation was conducted by trained professionals who received training from a single endocrinologist. Breast development in girls was assessed through visual inspection and palpation, while testicular volume was measured to evaluate genital development in boys using the Prader orchidometer. Each parameter was classified into five stages: 1 – prepubertal; 2, 3, and 4 – pubertal; and 5 – postpubertal. When discrepancies arose between breast or testicular development and pubic hair staging, the former was prioritised.

Ethics approval and consent to participate

All study phases complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki (29). The baseline and follow-up evaluations were approved by the University of Porto Medical School/ S. João Hospital Centre Ethics Committee, except the 13-year follow-up approved by the ISPUP Ethics Committee. At baseline and follow-up evaluations, all procedures were explained to participants, and informed consent was signed by one of the parents or legal guardians (at 13 years, it was also signed by the participants). The Data Protection National Commission additionally approved the baseline evaluation. The study follows the present EU General Data Protection Regulation under close supervision of the Data Protection Office of ISPUP.

Statistical analysis

Proportions were compared using the chi-square test, mean using the Student's t-test and median using the Mann–Whitney U test. The analyses were stratified by sex.

Factor scores was estimated using empirical Bayes methods from graded response models (GRM), which enables handling ordered polytomous variables (30). This model assumes that item discrimination is not equal across all items and that differences between each response category are not the same across all items. In the present study, the resulting factor scores – hereafter referred to as the global score of pubertal development – were derived from items of pubic hair and breast/genital development, evaluated at 10 and 13 years old (supplemental material, Figure S1 and Table S3).

The association between the global score of pubertal development and the participant's exact age at 10- and 13-year follow-up was evaluated using the linear regression model. According to the strongest coefficient found, the exact age at 10-year follow-up in girls and the exact age at 13-year follow-up in boys were the variables that explained better the differences in the global score of puberty (data not shown).

A linear regression model was used to test the associations between exposure to food contaminants, individually and combined, and pubertal development. Both cross-sectional and longitudinal practice of leisure physical activity and total energy intake were pointed as potential confounding variables and tested. Only the cross-sectional variables remained statistically significant, being those included in the final model. Thus, two models are presented in this paper. Model 1 was adjusted for the exact participants' age, maternal age, maternal education, and cross-sectional practice of leisure physical activity. Model 2 was adjusted for model 1 plus cross-sectional total energy intake (kcal). Moreover, the analysis were performed separately for each of the 10 different estimated values of AA and then combined using Rubin's rules (31).

All statistical analyses were performed with a 5% significance level, and missing data were treated at random. The R software version 4.2.1 for Windows was used.

Results

Sociodemographic characteristics between eligible participants and the remaining cohort at baseline were similar (supplemental material, Table S1). Of the included participants, 51.3% were male, and 48.7% were female. Statistical differences were only found for maternal characteristics.

Regarding the general characteristics of the eligible participants (Table 1), no significant differences were found between girls and boys for maternal age and maternal education. However, some significant differences were found in the other variables. As expected, compared to girls, boys presented a higher level of physical activity, higher total energy intake, and higher z-BMI. Regarding the daily dietary exposure to AA and BPA at 4-, 7-, 10- and 13- years old, the median dietary exposure is higher in boys for both AA and BPA, decreasing with age in both sexes.

Table 2 presents the pubertal development of participants according to the Tanner scale at 10 and 13 years. The estimated percentage of participants in stage 1 of breast or genital rating was 26.6% in girls and 73.4% in boys at 10 years, while at 13 years, these percentages reduced, as expected, to 1.6% and 1.2%, respectively. At 13 years of age, 55.6% of the girls and 61.7% of the boys have reached stage 4 or 5 for the breast or genital rating. Despite that, regarding pubic hair, 61.2% of the girls and 31.4% of the boys have reached stage 4 or 5.

Table 3 presents the linear regression models to test the association between food contaminants exposure, individually and combined, with the global score of pubertal development. After adjusting for the participant’s exact age, maternal age, maternal education, the practice of leisure physical activity and total energy intake (model 2), a significant negative association was found between individual AA exposure at 7 years (-0.007 (95% CI: -0.013, -0.002)), 10 years (-0.006 (95% CI: -0.010, -0.003)) and 13 years (-0.005 (95% CI: -0.009, -0.001)) and a higher pubertal development global score in girls. On the contrary, there was a significant positive association for individual AA exposure at 13 years (0.003 (95% CI: 0.000, 0.007)) in boys. Regarding individual BPA exposure, a significant positive association with a higher pubertal development global score was found only in boys for exposures at 10 and 13 years of age. Testing the combined exposure in the model did not significantly change the results observed for the

individual exposure to each food contaminant, except for the AA association in boys that lost its significance.

Discussion

Considering data from a population-based birth cohort, the present study found an adverse effect of food contaminants exposure, namely acrylamide and bisphenol A, on pubertal development. In girls, higher exposure to AA at 7, 10 and 13 years of age was inversely associated with pubertal development at 10 and 13 years. In boys, higher exposure to AA at 13 years and higher exposure to BPA at 10 and 13 years of age was positively associated with precious pubertal development. The combined effect of these food contaminants was also tested, and the results remained the same, except for the effect of AA among boys, which lost its significance.

To the best of our knowledge, this is the first study assessing the association between food contaminants exposure, estimated through collected dietary information, and pubertal development, measured through the Tanner scale. Moreover, this study followed a prospective and cross-sectional epidemiological design to the extent that the exposure was measured before or at the same time as the outcome was assessed. In this work, we only found one significant negative longitudinal association between higher exposure to AA at 7 years and later pubertal development in girls.

According to studies performed in animal models, it was plausible that AA and BPA exposure causes later puberty by disrupting the hypothalamic-pituitary-gonadal axis, reducing sex hormone concentrations in both female and male animals (32,33). On the other hand, BPA also has estrogen-mimicking properties, which may trigger early puberty in both sexes (33).

The previous epidemiological evidence on the association between AA and BPA dietary exposure and impaired pubertal development in humans is limited and contradictory. The comparison of the present findings with other studies is also difficult, considering the methodological differences across them. First, most previous studies estimated the exposure in spot urine or blood samples. From what we know, only one study performed in preschool-age Japanese children in 2006 estimated dietary acrylamide exposure using 3-day diet records (34). Second, the moment of exposure assessment differs, which sometimes reflects

the pre-natal exposure and other times reflects the post-natal exposure, especially for BPA-tested associations. Third, different methodologies to assess pubertal development were used across studies, i.e., using other scales rather than the Tanner scale, the interpretation of one single parameter or an overall pubertal development score, and measuring sex hormones in the blood to determine the pubertal stage. Fourth, the cross-sectional design of most studies and the small sample size. Fifth, the variability of the chosen potential confounders also differs, especially concerning BMI and total energy intake. Given the conceptual framework of this study and the potential role of AA and BPA as an endocrine disruptor, our final model was adjusted for sociodemographic characteristics, lifestyle factors, and total energy intake. Since participants' BMI could mediate the observed association, any adjustment or stratification to the model for this variable would be inappropriate.

Regarding AA, in line with our results, in a study involving 230 boys and 198 girls aged 3–6 years, a positive association between dietary AA exposure and higher sex hormone levels was found exclusively in boys (34). A cross-sectional study using data from individuals aged 6-19 years of NHANES 2013-2016 found a negative correlation between blood acrylamide and glycidamide biomarkers and sex hormone levels in girls (32). However, contrary to our findings, these authors also found a significant negative association among boys (32). Another recent Japanese study among adolescents aged 13-14 years found that AA measured in urine was associated with lower testosterone levels and a delayed puberty stage in males (35). Finally, other authors did not find significant associations between blood acrylamide and glycidamide biomarkers and sex hormones using cross-sectional data from NHANES 2003-2004 of participants older than 12 years (36). Nevertheless, all these studies adjusted their models to BMI, among other potential confounders, which may conditionate the interpretation and the comparison of the results.

For BPA cross-sectional (37–39) and longitudinal (40–42) studies did not find an association between urinary BPA and abnormalities in pubertal development in girls, which is in line with our results. In another cross-sectional study from the United Kingdom, although concentrations of urinary BPA have shown a positive association with the onset of thelarche, the authors failed to find a statistical significance for the hazard ratio (43). One South Korean study found that prenatal

exposure to BPA was associated with early puberty but did not find a significant result considering childhood exposure (19). Additionally, a systematic review with meta-analysis, including only studies performed with female participants, found no significant association between BPA exposure and pubertal development (17). Among boys, aligned with the findings of this paper, a cross-sectional study performed among Chinese boys aged 9-18 found a significant positive association between urinary BPA and early pubertal onset (44). Still, contradictory to our results, some authors found positive associations with statistical significance between BPA exposure and precocious puberty in girls (45–47). In boys, several studies failed to find a significant association (46,48). In one cohort study, including 250 Mexican boys, prenatal BPA exposure was associated with reduced odds of adrenarche and puberty. Still, no clear association was found between childhood BPA exposure and pubertal onset (49). There are some possible explanations for these differences. As observed for AA studies, most of the previously published results were adjusted for BMI, making direct comparison difficult, as the effect of food contaminants exposure on pubertal development may not be accurately measured in the adjusted model. Furthermore, the dual functionality of BPA, acting as an agonist or antagonist in the causal pathway, may explain the contradictory results of the BPA effect on early or later pubertal development.

It is also important to highlight that spot or first-morning urines may not correctly represent exposure to food contaminants, especially for those with relatively short half-lives, such as AA and BPA. In fact, the half-live ranges between 5 to 8 hours for AA (50) and approximately 6 hours for BPA body (51). Furthermore, the intra-individual variability and the inconstant concentration of food contaminants in urine throughout the day may not be captured with a single sample collection (48,52).

Only two cited studies assessed the effect of childhood combined exposures (phthalates and phenols) (19,53). They failed to find a significant association with impaired pubertal development in girls and boys. Our findings revealed that significant associations of AA and BPA exposure (cross-sectionally and longitudinally) with abnormalities in the timing of puberty onset remained significant when combined exposure to these food contaminants was tested. This

represents an important input to fill the gap in knowledge since it provides a more realistic perspective from real-world exposure.

Lastly, the interpretation of a single pubertal parameter appears limited, considering the different velocities of pubic hair versus breast/genital rating in girls and boys (27,28). Additionally, we cannot discard some misclassification in our sample, especially in boys. Puberty typically begins in boys about two years later than in girls, and many boys aged 10 to 13 are still prepubescent (28). However, boys in our cohort appear to experience a relatively rapid pace of pubertal development, progressing in genital stages compared to pubic hair within this age range. This justified the use of GRM to compute a global score of pubertal development, including the most relevant and discriminating parameters. We concluded from the reliability analysis (supplemental material, Table S2) that the high missing rate for axillary hair might indicate issues with this variable, conditioning its relevance to the model. Therefore, axillary hair was not included in the calculated global score of pubertal development.

Limitations and strengths

Our analyses have some limitations that must be addressed. First, the sociodemographic characteristics of the eligible participants may suggest a potential presence of participation bias. However, given that statistically significant results were found in our sample for maternal characteristics, we hypothesise that the effects observed in the general population would be even more pronounced. Second, a possible misclassification of the pubertal stage, especially in boys, was detected. We try to overcome this limitation by computing a global score of pubertal development using a empirical Bayes method from graded response model, as explained above. Third, we opted for a linear regression model since our outcome variables did not allow us to apply a mathematical longitudinal model (only two outcome observations). However, since the exposure was measured before the outcome, we were able to interpret the results longitudinally from an epidemiologic perspective.

Nonetheless, this study had several strengths. First, the use of a prospective population-based birth cohort with extensive data collection on sociodemographic characteristics, food consumption, and anthropometric and pubertal measurements. Second, the study includes a large sample size of eligible

participants, enhancing its statistical power. Third, a large comprehensive database with dietary information was used to estimate exposure to food contaminants. Fourth, the AA exposure assessment accounted for inter-individual and intra-individual variability. Fifth, the availability of 24-hour urine samples for a sub-sample of the GXXI cohort enabled a more accurate estimation of total daily BPA exposure. In fact, when combined with dietary data, this information allowed the construction of a random forest model, an approach rarely employed in previous research. Finally, the development of a global score of pubertal development, which integrated the different Tanner parameters, and enabled a more accurate interpretation of results by reducing errors of classification.

In the past years, concerns regarding the potential effect of EDC exposure on adverse health outcomes related to puberty development have been raised (1,4,54). There is a plausible causal relation between the dysregulation of the sexual hormone system caused by dietary exposure to EDC, such as acrylamide and BPA, responsible for the increased adverse health outcomes and burden of diseases in future life stages. The findings of the present study highlight the importance of reducing daily exposure to both acrylamide and BPA in children and adolescents, as chronic intake was associated with adverse health effects, namely impaired puberty onset. We hope this knowledge will support public health researchers, food safety authorities, and policymakers to design updated guidelines, recommendations, and food policies to mitigate these risks.

Conclusion

Childhood and adolescent exposure to acrylamide and bisphenol A was associated with impaired timing of puberty onset. Higher exposure to acrylamide at 7, 10 and 13 years of age was negatively associated with pubertal development at 10 and 13 years in girls. A higher exposure to acrylamide at 13 years and a higher exposure to bisphenol A at 10 and 13 years were positively associated with precious pubertal development in boys. The combined exposure effect of acrylamide and bisphenol A did not significantly change the found associations. Further studies applying longitudinal models are needed to assess the exposure

effects of these food contaminants on pubertal development and reduce the uncertainties surrounding these associations.

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Data availability

The data from Generation XXI are not publicly available due to privacy or ethical restrictions. The data can be made available for research proposals to the Generation XXI Executive Committee (generationxxi@ispup.up.pt) upon request. Further information about Generation XXI can be obtained via the Generation XXI website [www.geracao21.com] or by emailing generationxxi@ispup.up.pt.

Disclosure statement

The authors have no competing interests to declare.

Contribute of authors

SAC, CL and DT contributed to the design and implementation of the research. MS, CCS and SAC to the analysis of the results.

SAC wrote the original draft manuscript. SAC, MS, CCS, CL and DT reviewed the manuscript.

References

1. Uldbjerg CS, Koch T, Lim YH, Gregersen LS, Olesen CS, Andersson AM, et al. Prenatal and postnatal exposures to endocrine disrupting chemicals and timing of pubertal onset in girls and boys: a systematic review and meta-analysis. Hum Reprod Update. 2022 Aug;28(5):687–716.

2. Lopez-Rodriguez D, Franssen D, Heger S, Parent AS. Endocrine-disrupting chemicals and their effects on puberty. Best Pract Res Clin Endocrinol Metab [Internet]. 2021;35(5):101579. Available from: https://www.sciencedirect.com/science/article/pii/S1521690X21000968

3. Manyori F, Sun H, Yan W, Wu D, Xia Y, Wang X, et al. Association between phenols exposure and earlier puberty in children : A systematic review and meta-analysis. Environ Res. 2020;190(August):1–10.

4. Matoso V, Bargi-Souza P, Ivanski F, Romano MA, Romano RM. Acrylamide: A review about its toxic effects in the light of Developmental Origin of Health and Disease (DOHaD) concept. Food Chem. 2019 Jun;283:422–30.

5. Parent AS, Franssen D, Fudvoye J, Gérard A, Bourguignon JP. Developmental variations in environmental influences including endocrine disruptors on pubertal timing and neuroendocrine control: Revision of human observations and mechanistic insight from rodents. Front Neuroendocrinol. 2015 Jul;38:12–36.

6. Murkovic M, Pedreschi F. Potentially Toxic Food Components Formed by Excessive Heat Processing. In: Uribarri J, editor. Dietary AGEs and Their Role in Health and Disease. 1st ed. Boca Raton, Florida: CRC Press; 2017. p. 87–101.

7. Bušová M, Bencko V, Veszelits Laktičová K, Holcátová I, Vargová M. Risk of exposure to acrylamide. Cent Eur J Public Health. 2020 Oct;28 Suppl:S43–6.

8. EFSA CONTAM Panel. Scientific Opinion on acrylamide in food. EFSA Journal. 2015;13(6).

9. Costa SA, Correia D, Carvalho C, Vilela S, Severo M, Lopes C, et al. Risk characterization of dietary acrylamide exposure and associated factors in the Portuguese population. Food Additives & Contaminants: Part A [Internet]. 2022 Mar 14;1–13. Available from: https://doi.org/10.1080/19440049.2022.2047540

10. International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Industrial Chemicals. Vol. 60. Lyon; 1994.

11. Sto M, Matavulj M, Markovi J. Subchronic exposure to acrylamide leads to pancreatic islet remodeling determined by alpha cell expansion and beta cell mass reduction in adult rats. Acta Histochem. 2018;120(February):228–35.

12. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons W V. Human exposure to bisphenol A (BPA). Reproductive Toxicology. 2007;24(2):139–77.

13. European Food Safety Authority. Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA Journal [Internet]. 2023 Apr 1;21(4):e06857. Available from: https://doi.org/10.2903/j.efsa.2023.6857

14. Kang JH, Kondo F, Katayama Y. Human exposure to bisphenol A. Toxicology [Internet]. 2006;226(2):79–89. Available from: https://www.sciencedirect.com/science/article/pii/S0300483X06004057

15. Liu J, Wattar N, Field CJ, Dinu I, Dewey D, Martin JW. Exposure and dietary sources of bisphenol A (BPA) and BPA-alternatives among mothers in the APrON cohort study. Environ Int [Internet]. 2018;119(March):319–26. Available from: https://doi.org/10.1016/j.envint.2018.07.001

16. Chang WH, Liu SC, Chen HL, Lee CC. Dietary intake of 4-nonylphenol and bisphenol A in Taiwanese population: Integrated risk assessment based on probabilistic and sensitive approach. Environmental Pollution. 2019 Jan 1;244:143–52.

17. Bigambo FM, Sun H, Yan W, Wu D, Xia Y, Wang X, et al. Association between phenols exposure and earlier puberty in children: A systematic review and meta-analysis. Environ Res. 2020 Nov;190:110056.

18. Giulivo M, Lopez de Alda M, Capri E, Barceló D. Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. A review. Environ Res [Internet]. 2016;151:251–64. Available from: https://www.scopus.com/inward/record.uri?eid=2-s2.0-84982863801&doi=10.1016%2Fj.envres.2016.07.011&partnerID=40&md5=a13440caf591f18cb10588ebe3c73b87

19. Choe Y, Kim K nam, Jeong Y, Inhyang J, Kim B nyun, Lim Y hee, et al. Prenatal and childhood exposure to endocrine-disrupting chemicals and early thelarche in 8-year-old girls : A prospective study using Bayesian kernel regression. Environ Res [Internet]. 2024;263(P1):120056. Available from: https://doi.org/10.1016/j.envres.2024.120056

20. Larsen PS, Kamper-Jørgensen M, Adamson A, Barros H, Bonde JP, Brescianini S, et al. Pregnancy and birth cohort resources in Europe: A large opportunity for aetiological child health research. Vol. 27, Paediatric and Perinatal Epidemiology. Blackwell Publishing Ltd; 2013. p. 393–414.

21. US Department of Agriculture Agricultural Research Service. USDA National Nutrient Database for Standard Reference: Nutrient Data Laboratory. www.ars.usda.gov. 2004.

22. Lopes C, Torres D, Oliveira A, Severo M, Guiomar S, Alarcão V, et al. National food, nutrition, and physical activity survey of the Portuguese general population (2015-2016): Protocol for design and development. J Med Internet Res [Internet]. 2018;20(2):1–11. Available from: www.ian-af.up.pt

23. Goios ACL, Severo M, Lloyd AJ, Magalhães VPL, Lopes C, Torres DPM. Validation of a new software eAT24 used to assess dietary intake in the adult Portuguese population. Public Health Nutr. 2020;(3):1–11.

24. Costa SA, Severo M, Correia D, Carvalho C, Magalhães V, Vilela S, et al. Methodological approaches for the assessment of bisphenol A exposure. Food Research International [Internet]. 2023;173:113251. Available from: https://www.sciencedirect.com/science/article/pii/S0963996923007962

25. Guerra RS, Fonseca I, Pichel F, Restivo MT, Amaral TF. Hand length as an alternative measurement of height. Eur J Clin Nutr. 2014;68(2):229–33.

26. WHO Multicentre Growth Reference Study Group. Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age Methods and development. Geneva; 2006.

27. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969 Jun;44(235):291–303.

28. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child. 1970 Feb;45(239):13–23.

29. World Medical Association. WMA Declaration of Helsinki: ethical principles for medical research involving human subjects. 1974;353(1):1418–9. Available from: http://www.wma.net/en/30publications/10policies/b3/index.html

30. Samejima F. Estimation of latent ability using a response pattern of graded scores. Psychometrika Monograph Supplement. 1969;34(4, Pt. 2):100.

31. Marshall A, Altman DG, Holder RL, Royston P. Combining estimates of interest in prognostic modelling studies after multiple imputation: current practice

and guidelines. BMC Med Res Methodol [Internet]. 2009;9(1):57. Available from: https://doi.org/10.1186/1471-2288-9-57

32. Yu Y, Zhang D, Xu J, Zhang D, Yang L, Xia R, et al. Adolescence is a sensitive period for acrylamide-induced sex hormone disruption: Evidence from NHANES populations and experimental mice. Ecotoxicol Environ Saf. 2023 Jan;249:114413.

33. Calcaterra V, Cena H, Loperfido F, Rossi V, Grazi R, Quatrale A, et al. Evaluating Phthalates and Bisphenol in Foods: Risks for Precocious Puberty and Early-Onset Obesity. Nutrients. 2024 Aug;16(16).

34. Nagata C, Konishi K, Wada K, Tamura T, Goto Y, Koda S, et al. Associations of Acrylamide Intake With Urinary Sex Hormone Levels Among Preschool-Age Japanese Children. Am J Epidemiol. 2018 Jan;187(1):75–81.

35. Nagata C, Wada K, Yamakawa M, Sugino M, Mori T, Ueyama J, et al. Acrylamide exposure, sex hormones, and pubertal status in Japanese adolescents. Int J Environ Health Res. 2024 Sep;1–10.

36. Chu PL, Liu HS, Wang C, Lin CY. Association between acrylamide exposure and sex hormones in males: NHANES, 2003–2004. PLoS One [Internet]. 2020 Jun 18;15(6):e0234622. Available from: https://doi.org/10.1371/journal.pone.0234622

37. Jung MK, Choi HS, Suh J, Kwon A, Chae HW, Lee WJ, et al. The analysis of endocrine disruptors in patients with central precocious puberty. BMC Pediatr. 2019 Sep;19(1):323.

38. McGuinn LA, Ghazarian AA, Joseph Su L, Ellison GL. Urinary bisphenol A and age at menarche among adolescent girls: evidence from NHANES 2003-2010. Environ Res. 2015 Jan;136:381–6.

39. Buluş AD, Aşci A, Erkekoglu P, Balci A, Andiran N, Koçer-Gümüşel B. The evaluation of possible role of endocrine disruptors in central and peripheral precocious puberty. Toxicol Mech Methods. 2016 Sep;26(7):493–500.

40. Wolff MS, Pajak A, Pinney SM, Windham GC, Galvez M, Rybak M, et al. Associations of urinary phthalate and phenol biomarkers with menarche in a multiethnic cohort of young girls. Reprod Toxicol. 2017 Jan;67:56–64.

41. Wolff MS, Teitelbaum SL, McGovern K, Pinney SM, Windham GC, Galvez M, et al. Environmental phenols and pubertal development in girls. Environ Int. 2015 Nov;84:174–80.

42. Wolff MS, Teitelbaum SL, Pinney SM, Windham G, Liao L, Biro F, et al. Investigation of relationships between urinary biomarkers of phytoestrogens, phthalates, and phenols and pubertal stages in girls. Environ Health Perspect. 2010 Jul;118(7):1039–46.

43. Howland RE, Deziel NC, Bentley GR, Booth M, Choudhury OA, Hofmann JN, et al. Assessing Endogenous and Exogenous Hormone Exposures and Breast Development in a Migrant Study of Bangladeshi and British Girls. Int J Environ Res Public Health. 2020 Feb;17(4).

44. Wang Z, Li D, Miao M, Liang H, Chen J, Zhou Z, et al. Urine bisphenol A and pubertal development in boys. Int J Hyg Environ Health [Internet]. 2017;220(1):43–50. Available from: https://www.sciencedirect.com/science/article/pii/S1438463916304254

45. Durmaz E, Aşçı A, Erkekoğlu P, Akçurin S, Koçer Gümüşel B, Bircan İ. Urinary Bisphenol A Levels in Girls with Idiopathic Central Precocious Puberty. J Clin Res Pediatr Endocrinol [Internet]. 2014;6(1):16–21. Available from: https://dx.doi.org/10.4274/Jcrpe.1220

46. Kasper-Sonnenberg M, Wittsiepe J, Wald K, Koch HM, Wilhelm M. Pre-pubertal exposure with phthalates and bisphenol A and pubertal development. PLoS One. 2017;12(11):e0187922.

47. Chen Y, Wang Y, Ding G, Tian Y, Zhou Z, Wang X, et al. Association between bisphenol a exposure and idiopathic central precocious puberty (ICPP) among school-aged girls in Shanghai, China. Environ Int [Internet]. 2018;115:410–6. Available from: https://www.sciencedirect.com/science/article/pii/S0160412017316689

48. Frederiksen H, Aksglaede L, Sorensen K, Nielsen O, Main KM, Skakkebaek NE, et al. Bisphenol A and other phenols in urine from Danish children and adolescents analyzed by isotope diluted TurboFlow-LC–MS/MS. Int J Hyg Environ Health [Internet]. 2013;216(6):710–20. Available from: https://www.sciencedirect.com/science/article/pii/S1438463913000163

49. Ferguson KK, Peterson KE, Lee JM, Mercado-García A, Blank-Goldenberg C, Téllez-Rojo MM, et al. Prenatal and peripubertal phthalates and bisphenol A in relation to sex hormones and puberty in boys. Reproductive Toxicology [Internet]. 2014;47:70–6. Available from: https://www.sciencedirect.com/science/article/pii/S0890623814001038

50. Miller MJ, Carter DE, Sipes IG. Pharmacokinetics of acrylamide in Fisher-344 rats. Toxicol Appl Pharmacol. 1982 Mar;63(1):36–44.

51. Thayer KA, Doerge DR, Hunt D, Schurman SH, Twaddle NC, Churchwell MI, et al. Pharmacokinetics of Bisphenol A in Humans Following a Single Oral Administration HHS Public Access. Environ Int. 2015;83:107–15.

52. Pollack AZ, Perkins NJ, Sjaarda L, Mumford SL, Kannan K, Philippat C, et al. Variability and exposure classification of urinary phenol and paraben metabolite concentrations in reproductive-aged women. Environ Res. 2016 Nov 1;151:513–20.

53. Freire C, Castiello F, Babarro I, Anguita-ruiz A, Casas M, Vrijheid M, et al. International Journal of Hygiene and Environmental Health Association of prenatal exposure to phthalates and synthetic phenols with pubertal development in three European cohorts. Int J Hyg Environ Health. 2024;261(February).

54. European Environment Agency. The impacts of endocrine disrupters on wildlife, people and their environments. Publications Office of the European Union, editor. Luxembourg; 2012.

Tables

Table 1: General characteristics of the eligible participants, stratified by sex.

	Girls (n = 2572)	**Boys** (n = 2707)	**p-value**
Maternal age (years), *mean (SD)*	30.0 (6.3)	29.7 (6.2)	0.108
Maternal education (years), *mean (SD)*	11.1 (4.3)	11.2 (4.3)	0.407
Physical Activity[a], *n (%)*			
4 years old	1788 (70.7)	1797 (67.9)	0.078
7 years old	1660 (71.6)	1691 (68.8)	0.101
10 years old	1415 (59.2)	1720 (68.1)	**<0.001**
13 years old	989 (51.2)	1362 (67.1)	**<0.001**
Total Energy Intake (kcal), *mean (SD)*			
4 years old	1562 (280.8)	1641 (301.6)	**<0.001**
7 years old	1711 (293.6)	1812 (313.4)	**<0.001**
10 years old	1855 (364.3)	1947 (396.1)	**<0.001**
13 years old	1759 (405.3)	1923 (445.9)	**<0.001**
z-BMI, *mean (SD)*			
4 years old	0.62 (1.10)	0.56 (1.06)	0.061
7 years old	0.71 (1.14)	0.70 (1.22)	0.728
10 years old	0.63 (1.21)	0.74 (1.25)	**0.002**
13 years old	0.47 (1.13)	0.41 (1.22)	0.076
Acrylamide, µg/kg bw/day, *median (P25-P75)*			
4 years old	0.76 (0.50, 1.11)	0.83 (0.56, 1,23)	**<0.001**
7 years old	0.63 (0.42, 0.89)	0.67 (0.45, 0.94)	**<0.001**
10 years old	0.58 (0.39, 0.85)	0.62 (0.41, 0.90)	**0.002**
13 years old	0.41 (0.27, 0.60)	0.46 (0.30, 0.66)	**<0.001**
Bisphenol A, ng/kg bw/day, *median (P25-P75)*			
4 years old	69.3 (60.7, 79.0)	68.5 (61.1, 76.0)	**0.03**
7 years old	50.2 (42.6, 57.8)	50.1 (43.8, 57.1)	0.81
10 years old	35.7 (29.7, 41.9)	36.8 (30.5, 42.4)	**0.006**
13 years old	24.4 (20.8, 28.4)	25.1 (21.0, 29.6)	**0.002**

Abbreviations: n – sample size, % – percentage, SD – standard deviation, kcal – kilocalories, z-BMI – body mass index z-score, µg – microgram, ng – nanogram, kg – kilogram, bw – body weight, P25 – 25th percentile, P75 – 75th percentile.

Notes: a) Practice of leisure physical activity outside the school setting. p-values from the chi-square test, Student's t-test or Mann–Whitney U test, as appropriate.

Significant values are in **bold**.

Table 2: Pubertal development of participants at 10- and 13-years old, stratified by sex.

	Girls		**Boys**	
	n	%	n	%
Pubic hair, 10 years				
Stage 1	984	45.8	1925	85.3
Stage 2	689	32.0	278	12.3
Stage 3	394	18.3	44	2.0
Stage 4	80	3.7	8	0.4
Stage 5	3	0.1	1	0.0
Breast or genital rating[a], 10 years				
Stage 1	574	26.6	1658	73.4
Stage 2	986	45.8	547	24.2
Stage 3	501	23.3	45	2.0
Stage 4	93	4.3	8	0.4
Stage 5	0	0.0	1	0.0
Axillary hair, 13 years				
Stage 1	111	22.6	746	39.0
Stage 2	170	34.6	528	27.6
Stage 3	128	26.0	406	21.2
Stage 4	69	14.0	195	10.2
Stage 5	14	2.8	37	1.9
Pubic hair, 13 years				
Stage 1	9	0.5	261	13.3
Stage 2	99	5.9	489	25.0
Stage 3	543	32.3	592	30.3
Stage 4	857	51.0	513	26.2
Stage 5	172	10.2	102	5.2
Breast or genital rating[a], 13 years				
Stage 1	32	1.6	25	1.2
Stage 2	209	10.7	262	13.1
Stage 3	706	36.2	481	24.0
Stage 4	850	43.6	715	35.7
Stage 5	152	7.8	521	26.0

Abbreviations: n – sample size, % – percentage.

Notes: a) Breast rating for girls or genital rating for boys.

Table 3: Association between acrylamide (µg/day) and bisphenol A (ng/day) exposure and the global score of pubertal development applying linear regression models, stratified by sex (β and 95% confidence interval (CI)).

	Girls		**Boys**	
	Model 1	**Model 2**	**Model 1**	**Model 2**
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
AA				
4 years old	0.001 (-0.005, 0.007)	-0.001 (-0.008, 0.005)	**0.006 (0.000, 0.012)**	0.002 (-0.004, 0.008)
7 years old	-0.004 (-0.009, 0.001)	**-0.007 (-0.013, -0.002)**	0.003 (-0.001, 0.008)	0.001 (-0.004, 0.007)
10 years old	**-0.005 (-0.008, -0.001)**	**-0.006 (-0.010, -0.003)**	0.000 (-0.003, 0.004)	0.000 (-0.004, 0.004)
13 years old	**-0.005 (-0.008, -0.001)**	**-0.005 (-0.009, -0.001)**	**0.004 (0.001, 0.007)**	**0.003 (0.000, 0.007)**
BPA				
4 years old	0.02 (-0.48, 0.52)	0.02 (-0.48, 0.52)	0.35 (-0.18, 0.88)	0.33 (-0.19, 0.86)
7 years old	-0.16 (-0.59, 0.27)	-0.15 (-0.58, 0.28)	0.13 (-0.31, 0.57)	0.17 (-0.26, 0.61)
10 years old	-0.07 (-0.45, 0.31)	-0.07 (-0.45, 0.31)	**0.46 (0.03, 0.88)**	**0.46 (0.04, 0.88)**
13 years old	-0.12 (-0.46, 0.22)	-0.13 (-0.47, 0.21)	**0.47 (0.14, 0.81)**	**0.49 (0.15, 0.82)**
AA+BPA				
4 years old, AA	0.000 (-0.005, 0.005)	-0.001 (-0.007, 0.004)	0.005 (-0.001, 0.010)	0.002 (-0.004, 0.008)
4 years old, BPA	0.02 (-0.48, 0.52)	0.03 (-0.48, 0.53)	0.33 (-0.20, 0.86)	0.33 (-0.20, 0.86)
7 years old, AA	-0.004 (-0.009, 0.001)	**-0.007 (-0.013, -0.002)**	0.003 (-0.001, 0.008)	0.001 (-0.004, 0.006)
7 years old, BPA	-0.15 (-0.58, 0.28)	-0.13 (-0.56, 0.30)	0.11 (-0.33, 0.55)	0.16 (-0.28, 0.60)
10 years old, AA	**-0.004 (-0.007, -0.001)**	**-0.005 (-0.009, -0.002)**	0.000 (-0.004, 0.003)	0.000 (-0.004, 0.003)
10 years old, BPA	-0.08 (-0.46, 0.30)	-0.07 (-0.45, 0.31)	**0.46 (0.03, 0.88)**	**0.46 (0.04, 0.88)**
13 years old, AA	**-0.004 (-0.008, -0.001)**	**-0.004 (-0.008, 0.000)**	**0.003 (0.000, 0.006)**	0.002 (-0.001, 0.006)
13 years old, BPA	-0.13 (-0.47, 0.21)	-0.13 (-0.47, 0.21)	**0.47 (0.13, 0.81)**	**0.48 (0.14, 0.82)**

Abbreviations: β – regression coefficient, 95% CI – 95% confidence interval, AA – acrylamide; BPA – bisphenol A.

Model 1: adjusted for participant's exact age (at 10-year follow-up for girls and at 13-year follow-up for boys), maternal age, maternal education, and cross-sectional practice of leisure physical activity. Model 2: adjusted for model 1 plus cross-sectional total energy intake (kcal).

Significant values are in **bold**.

Supplemental material

Table S1: Sociodemographic characteristics at baseline of the eligible participants and those of the remaining cohort.

	Eligible participants (n = 5279)	Remaining cohort (n = 3368)	p-value
Maternal age (years)*, mean (SD)*	29.8 (5.2)	27.8 (5.9)	**<0.001**
Maternal education (years)*, mean (SD)*	11.2 (4.3)	9.3 (4.0)	**<0.001**
Maternal smoking habits*, n (%)*			**<0.001**
Smokers	1774 (33.8)	1481 (44.3)	
Non-smokers	3475 (66.2)	1864 (55.7)	
Child sex*, n (%)*			0.53
Female	2572 (48.7)	1665 (49.4)	
Male	2707 (51.3)	1703 (50.6)	
Childbirth weight (g), *mean (SD)*	3146.3 (533.41)	3152.3 (545.69)	0.61
Childbirth length (cm), *mean (SD)*	48.61 (2.54)	48.6 (2.57)	0.89

Abbreviations: n – sample size, % – percentage, SD – standard deviation.

Note: p-values from chi-square test or Student's t test, as appropriate.

Significant values are in **bold**.

Table S2: Reliability analysis of Tanner scale parameters.

	α	med.r	r.drop	% miss
Girls				
Pubic hair, 10 years	0.72	0.38	0.48	40
Breast rating, 10 years	0.70	0.39	0.53	39
Axillary hair, 13 years	0.69	0.31	0.57	87
Pubic hair, 13 years	0.70	0.36	0.56	55
Breast rating, 13 years	0.73	0.36	0.46	47
Boys				
Pubic hair, 10 years	0.72	0.37	0.30	40
Genital rating, 10 years	0.69	0.33	0.43	45
Axillary hair, 13 years	0.58	0.30	0.63	50
Pubic hair, 13 years	0.59	0.33	0.62	49
Genital rating, 13 years	0.67	0.31	0.45	47

Abbreviations and notes

- α – raw alpha. Indicates the internal consistency to test if the items collectively measure a coherent construct.
- med.r – median of inter-item correlations. Represents the median of the correlation coefficients between a specific item and all the remaining items in the scale.
- r.drop – item-scale correlation after dropping the item. Measures the unique contribution of a specific item to the overall reliability of the scale
- % miss – miss rates, measured through percentage of missing reports.

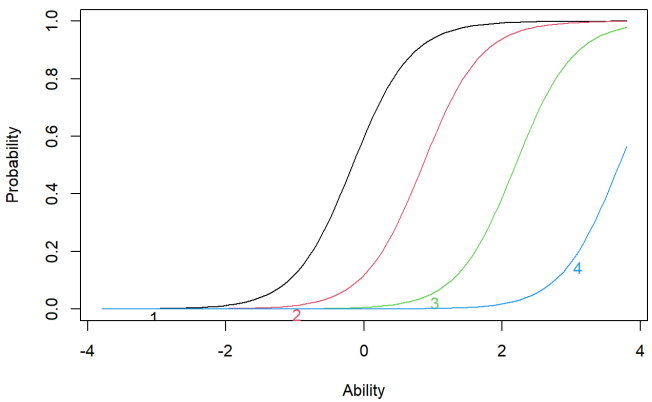
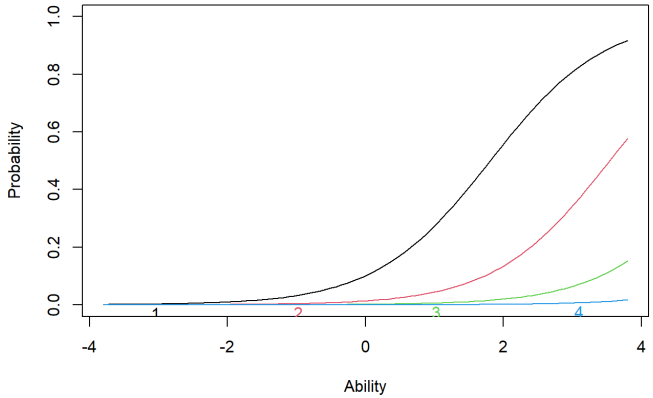
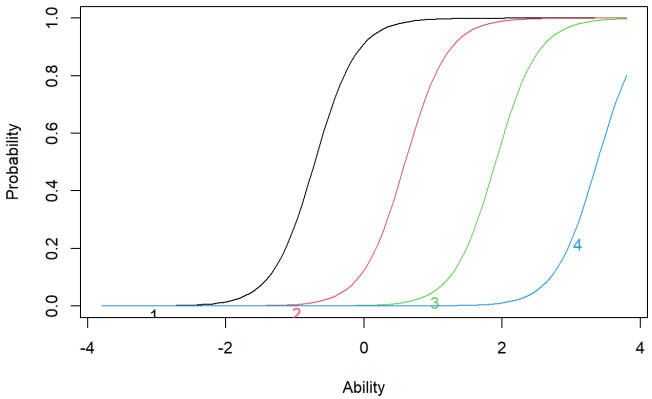
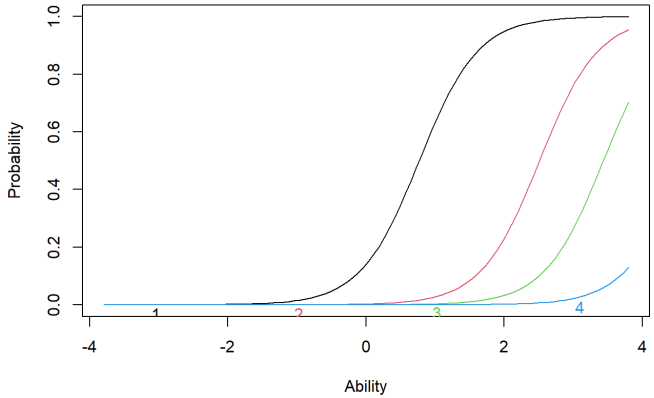
Table S3: Graded response model item parameter estimates.

	a	b1	b2	b3	b4
Girls					
Pubic hair, 10 years	2.374	-0.166	0.848	2.191	3.689
Breast rating, 10 years	3.273	-0.715	0.595	1.903	3.374
Pubic hair, 13 years	1.039	-5.380	-3.144	-0.630	2.436
Breast rating, 13 years	0.961	-4.999	-2.510	-0.151	2.845
Boys					
Pubic hair, 10 years	1.208	1.807	3.540	5.218	7.612
Genital rating, 10 years	2.360	0.769	2.511	3.435	4.604
Pubic hair, 13 years	1.246	-1.885	-0.478	0.815	2.785
Genital rating, 13 years	1.745	-3.316	-1.499	-0.397	0.892

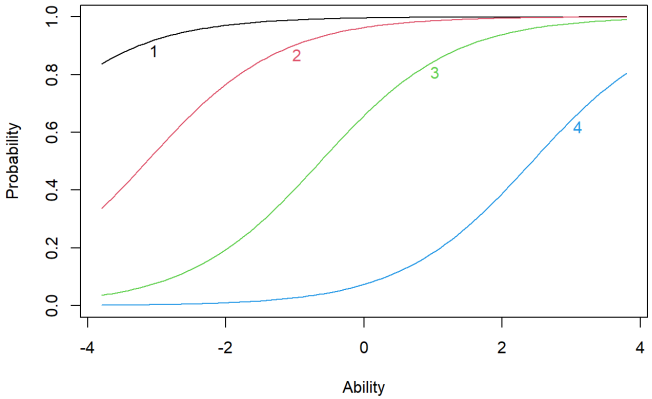
Abbreviations and notes

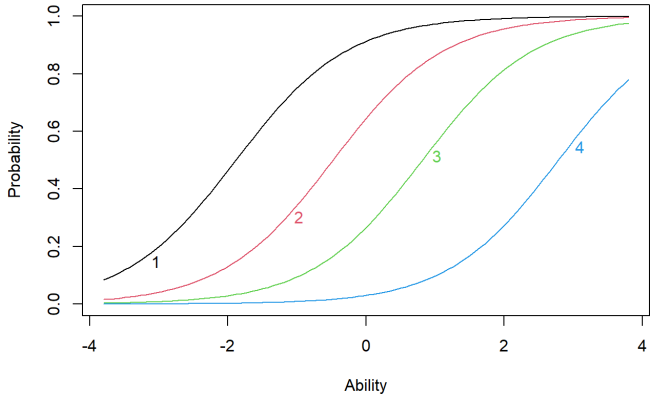
- a – item's discrimination (item slope).
- b – Item threshold (item difculty).

Figure S1: Item operation characteristic curves.

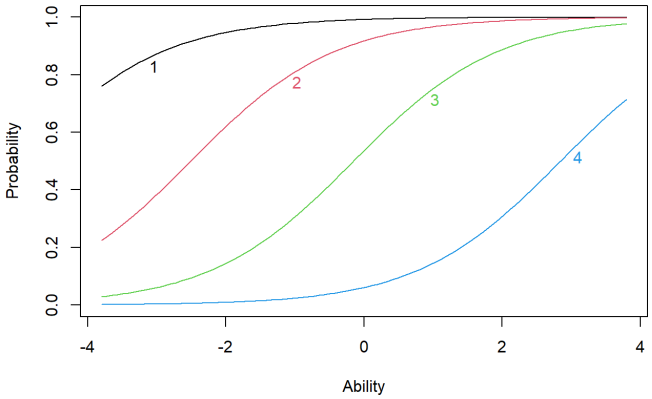
	Girls	Boys
Pubic hair, 10 years		
Breast or genital rating[a], 10 years		

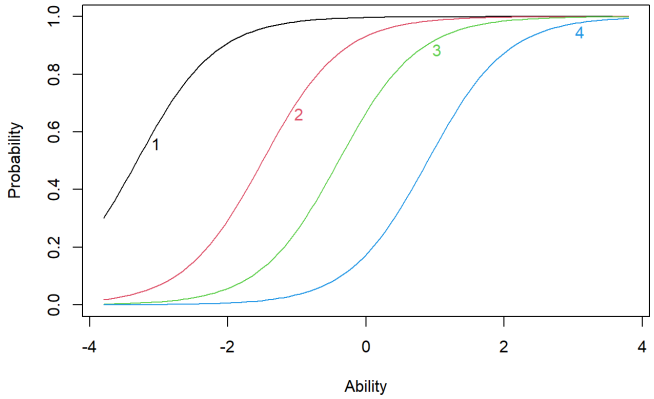
Pubic hair, 13 years





Breast or genital rating[a], 13 years





Notes: a) Breast rating for girls or genital rating for boys.

GENERAL DISCUSSION

In this thesis, we tested the association between food contaminants exposure and the development of adverse metabolic outcomes in children and adolescents, focusing the exposure assessment on acrylamide and bisphenol A. In this context, the effect of acrylamide and bisphenol A exposure on pubertal development was also assessed. For that, we tested different methodological approaches to estimate the daily dietary exposure to these food contaminants. Data from two large well-designed epidemiologic studies was used. The present work included evidence from the population-based birth cohort Generation XXI and from the National Food, Nutrition and Physical Activity Survey of the Portuguese population.

3.1. Summary of results

To ensure food safety and to protect human health, the most accurate scientific evidence is needed to answer questions about the risk. Food contaminants are scientifically analysed in a food safety risk assessment to determine their potential to cause adverse health effects due to human dietary exposure to these hazards. The process allows a comprehensive evaluation of the risks that consumers may face, functioning as the scientific input for decision-making by risk managers and politicians.

The methodology chosen to estimate the exposure assessment to food contaminants is essential, even more so if we are trying to capture the risk implicated in a real-world scenario. Taking this into consideration, we adopted a probabilistic methodology to assign the acrylamide occurrence values to each consumption occasion, and we evaluated the performance of different methodological approaches to estimate the exposure to BPA.

Using dietary data from IAN-AF 2015-2016, collected through self-reported assessment instruments, and considering the maximum available data published by EFSA on acrylamide occurrence in food, an alternative methodology was designed to estimate the daily dietary exposure [**Paper I**]. Here, instead of using a single average value, three possible values representing the distribution of acrylamide occurrence in foodstuffs were randomly attributed to each food item

reported in the IAN-AF 2015-2016. Furthermore, 10 cycles of random attributions were performed to increase the variability, handle the uncertainties and ensure the achievement of a more realistic scenario. We believe that this approach improves the predictions, widening the range of exposure estimates by accounting for the intra and inter-individual variability, usually underestimated in the deterministic models. This hypothesis was corroborated by previous studies in which authors concluded that probabilistic models are one of the most suitable approaches for designing new public health guidelines and for exposure management purposes (30,149). Besides, following a more conservative approach, we used an upper-bound methodology for the risk assessment to characterise the worst-case exposure scenario.

Thus, the estimated median dietary exposure to acrylamide for the general population was 0.38 µg/kg of body weight (bw)/day, with Portuguese children and adolescents being the age groups with higher acrylamide exposure and higher health concerns. Portuguese children aged 1 to 2 years old presented the higher median of dietary exposure, 0.75 µg/kg bw/day (95^{th} percentile: 1.41 µg/kg bw/day), followed by children of 3 to 9 years with 0.69 µg/kg bw/day (95^{th} percentile: 1.32 µg/kg bw /day) and adolescents of 10 to 17 years with 0.54 µg/kg bw/day (95^{th} percentile: 1.07 µg/kg bw /day). Regarding risk characterisation, for peripheral neuropathy, MOE values exceeded 125 for all percentiles of exposure and all age groups, indicating no public health concerns related to neurotoxicity in the Portuguese population. All MOE values were largely below 10,000 for neoplastic effects, regardless of the exposure percentile or age group, indicating a real public health concern for the Portuguese population. However, childhood and adolescence represent a critical window to hazard exposures, as they are still growing and changing, making these particularly vulnerable age groups of the population (150,151). Effectively, the higher exposure of food contaminants relative to body weight and the immature metabolic system or key organs underscores the importance of minimising exposure at this life stage to prevent the development of future adverse health outcomes.

These results were in accordance with previous studies. In France and Ireland, the dietary acrylamide exposure in the children was very similar to that in our study (152,153). A sample of French children aged 12 to 36 months had a mean

acrylamide exposure of 0.74 µg/kg bw/day (90th percentile:1.66 µg/kg bw/day) (152), and in Irish children, the mean of dietary acrylamide exposure was 0.71 µg/kg bw/day (97.5th percentile:1.42 µg/kg bw/day) (153). On the other hand, compared to EFSA exposure estimates for Europe, our assessment presented lower values of dietary acrylamide exposure. For toddlers, the exposure to acrylamide was 1.4 µg/kg bw/day (95th percentile: 2.4 µg/kg bw /day) across European surveys, while for other children and adolescents was 1.2 µg/kg bw/day (95th percentile: 2.3 µg/kg bw /day) and 0.7 µg/kg bw/day (95th percentile: 1.4 µg/kg bw /day), respectively (14). Nevertheless, direct comparison between studies is difficult due to differences in study designs and methods used to assess dietary intake. Usually, even considering lower and upper-bound scenarios, studies apply deterministic approaches, which may not represent the distribution of acrylamide exposure, especially in the population groups with extreme percentiles of consumption (149).

Finally, this paper identifies the associated factors and food contributors to acrylamide dietary exposure by age group. For Portuguese children and adolescents, the most important food groups for acrylamide exposure were bread, cookies and breakfast cereals, in line with previous findings in other countries. These results will be fundamental in the future for the definition and prioritisation of strategies to reduce contact with this food contaminant, targeting population settings, assessing the effectiveness of food policies and designing new food safety-health promotion interventions.

Given the diversity found by using different exposure assessment methods, we tested three methodological approaches to estimate daily BPA exposure [**Paper II**]. For that, we used data from self-reported dietary assessment methods and the urinary concentration of BPA. In the first method, the traditional deterministic approach, considering the upper-bound scenario, was followed to estimate the daily dietary exposure to BPA by merging the food consumption data with the occurrence data of BPA in food groups. In the second and third methods, dietary information, namely food groups and the packaging material reported, and urinary BPA concentration from a sub-sample were combined to develop a regression tree model and a random forest. Both methods were then used to

predict the daily exposure to BPA in the remaining sample. Our findings suggested that random forest was the best methodology to estimate the daily exposure to BPA.

Furthermore, the traditional approach proved less accurate in estimating daily BPA exposure, with median values in adolescents being 5 to 6 times higher than those measured in urine. Specifically, the median daily dietary BPA exposure in adolescents was 79.1 ng/kg bw/day using the traditional method, whereas the random forest approach yielded a lower value of 31.6 ng/kg bw/day. In addition to our traditional approach estimates not always aligning with previous studies (114,154,155), the findings suggest that this method may overestimate daily dietary BPA exposure compared to urinary measurements, raising concerns about the validity of this widely used methodology. Despite the methodological approach, 99.9% of the participants had exposure levels exceeding the TDI of 0.2 ng/kg bw/day (56), highlighting a potential public health concern among adolescents.

To the best of our knowledge, this was the first study testing three different methodological approaches to estimate daily BPA exposure. Addicitionally, this is a pioneer study that uses data from food consumption and 24-hour urinary excretion in the same methodological approach. Combining both information, we intend to develop a more precise and accurate model reflecting real-world exposure. Moreover, we demonstrated that by applying a random forest methodology the estimation of BPA daily exposure for the remaining sample or population is possible, even with a limited number of 24-hour urine samples. Previous research on human exposure to food contaminants, such as BPA, typically focused on traditional deterministic exposure assessments (i.e., food consumption merged with contaminant occurrence) or urinary measurements rather than integrating data from both direct and indirect methods. No studies evaluated the performance of different methodological approaches to estimate exposure to food contaminants, making it difficult to compare our results. Recently, a European study has shown that random forest outperforms linear regression in predicting serum vitamin D levels using dietary and lifestyle data (156). Besides that, random forest has been used in different settings, for example, to predict the serum concentrations of per- and polyfluoroalkyl

substances for women in late pregnancy considering individual food data, demographic parameters and serum fatty acids (157), to analyse trends in cadmium exposure based on environmental and socio-economic factors (158) or to test the parameters that influence the occurrence of arsenic in rice grains (159).

After defining the exposure assessment methodology, we longitudinally performed a risk assessment, considering data from the GXXI birth-cohort. Lastly, we tested the longitudinal association of food contaminants exposure with health outcomes from childhood to adolescence.

Our findings showed that the median dietary exposure to acrylamide ranged from 0.44 µg/kg bw/day at 13 years old (95^{th} percentile: 1.04 µg/kg bw/day) to 0.79 µg/kg bw/day at 4 years old (95^{th} percentile: 1.92 µg/kg bw/day) [**Paper III**]. The MOE values for peripheral neuropathy indicate no public health concern. However, for neoplastic effects, MOE was significantly below the safety threshold of 10,000 across all exposure levels and from childhood to adolescence, indicating a public health concern for genotoxic and carcinogenic risks among GXXI participants.

To the best of our knowledge, this was the first prospective study assessing the effects of acrylamide daily dietary exposure on cardiometabolic outcomes from childhood to adolescence. We found a positive longitudinal association between acrylamide exposure and higher waist circumference, fat mass percentage, and blood glucose levels. For blood insulin and insulin resistance, only a significant cross-sectional association was found. In the opposite direction, a negative longitudinal association was found for body mass index z-score, cholesterol, and blood pressure. Consistent with our study, different authors reported controversial results regarding the association between acrylamide exposure and cardiometabolic outcomes (90–93). Nevertheless, no studies have tested the relationship between food contaminant exposure and health outcomes during childhood and adolescence, with most of the previous results being performed in adults of the general population under cross-sectional designs. Moreover, this type of association was usually tested with the total acrylamide exposure

measured through blood biomarkers, such as haemoglobin adduct of acrylamide or haemoglobin adduct of glycidamide.

The variability in our results highlights the importance of interpreting these studies with caution. We may hypothesise that the effects of dietary acrylamide on cardiometabolic health are complex and may be influenced by multiple factors. In fact, it is widely accepted that the development of cardiometabolic disorders is related to multifactorial causes, including lifestyle behaviours, food consumption and environmental hazardous exposure (160). Thus, and despite the proper adjustment for possible confounders, it is not possible to conclude, without uncertainty, that dietary acrylamide exposure by itself is responsible for abnormalities in cardiometabolic outcomes from childhood to adolescence.

The absolute mean daily exposure to BPA ranged from 1229 to 1295 ng/day at 4 years and 10 years old, respectively [**Paper IV**], without significant differences, comparing eligible and non-eligible participants. At 4 years of age, the median BPA daily exposure was 68.8 ng/kg bw/day (95^{th} percentile: 90.6 ng/kg bw/day), while at 13 years old was 24.7 ng/kg bw/day (95^{th} percentile: 36.3 ng/kg bw/day). According to these exposure values, and assuming that food and beverages are the primary sources of BPA exposure in children and adolescents, 99.9% of the participants presented an exposure above the TDI of 0.2 ng/kg bw/day (56), which may indicate a public health concern at these ages.

Our study is one of the few prospective studies testing the association between BPA daily exposure (combining data from self-reported dietary assessment methods and urinary metabolites) and cardiometabolic outcomes, and the first performed from 4 to 13 years of age. A longitudinal positive association was found between BPA exposure and higher blood insulin levels, insulin resistance, fat mass percentage, waist circumference, and z-BMI. Our findings provided relevant scientific outputs to address some uncertainties raised in the past since previous studies failed to find a longitudinal association between exposure to BPA and cardiometabolic outcomes, at different time points of young ages (65,121,161,162). Additionally, these studies used spot urine samples to estimate BPA exposure, whereas our study combined data from 3-day food diaries and

BPA concentration in 24-hour urine samples to develop a random forest model to predict daily exposure to BPA. Lastly, we concluded that early BPA exposure may impair cardiometabolic outcomes later in life, highlighting the urgent need to reduce global daily exposure to this food contaminant.

Finally, the association of acrylamide and BPA exposure, individually and combined, on pubertal development was tested in children and adolescents aged 4 to 13 [**Paper V**]. In the scope of this study, pubertal development was assessed at ages 10 and 13 using the Tanner scale, a standardised method for evaluating sexual maturation. With these data, a global score of pubertal development at 10 and 13 years old was created using empirical Bayes method from graded response models. This model assumes that item discrimination is unequal across all items and that differences between each response category are not the same across all items. Thus, a global score of pubertal development was computed, encompassing the different items of the Tanner scale rather than focusing on a single item.

A negative association was found between individual acrylamide exposure at 7 years (-0.007 (95% CI: -0.013, -0.002)), 10 years (-0.006 (95% CI: -0.010, -0.003)) and 13 years (-0.005 (95% CI: -0.009, -0.001)) and a higher pubertal development global score in girls. On the contrary, there was a positive association for individual acrylamide exposure at 13 years (0.003 (95% CI: 0.000, 0.007)) in boys. Regarding individual BPA exposure, a significant positive association with a higher pubertal development global score was found only in boys for exposures at 10 and 13 years of age. Testing the combined exposure in the model did not significantly change the results observed for the individual exposure to each food contaminant.

To the best of our knowledge, this is the first study assessing the association between food contaminants exposure, estimated through collected dietary information, and a global score of pubertal development computed using the Tanner scale data. Moreover, this study followed a prospective and cross-sectional epidemiological design to the extent that the exposure was measured before or at the same time as the outcome was assessed. The previous

epidemiological evidence on the association between acrylamide and BPA dietary exposure and impaired pubertal development in humans is limited and contradictory. Some studies were in line with our results, showing a negative association between acrylamide exposure and pubertal development in girls, and a positive association of acrylamide and BPA exposure with pubertal development in boys (101,102,163). Moreover, aligned with our findings, a meta-analysis of studies including female participants found no significant association between BPA exposure and pubertal development (126). However, other authors did not find significant associations between exposure to acrylamide and impaired pubertal development (101,103,104); the same was observed for BPA exposure (128,164,165,165,166).

Considering the methodological differences, the comparison of the present findings with previous studies should be made with caution. Most of the studies estimated the exposure using spot urine or blood samples, which may not represent the usual chronic dietary exposure to these food contaminants. Many studies assessed pre-natal exposure rather than post-natal exposure, especially for BPA. Regarding pubertal development, most of the previous research explored one single parameter or measured sex hormones in the blood to determine the pubertal stage. Finally, the potential confounders chosen also differed across studies.

There is a plausible causal relationship between the dysregulation of pubertal development due to exposure to acrylamide and BPA, and our findings further support this theory, underscoring the importance of reducing daily exposure to these compounds in children and adolescents.

3.2. Limitations and strengths

The thesis presents some potential limitations that cannot be disclosed. First, the eligible participants of the GXXI cohort may have introduced some selection bias. The mothers in our sample were slightly older and more educated compared to the remaining participants. However, Cohen's effect size values in each paper were relatively low, indicating that these differences were more likely attributable

to the large sample size rather than relevant differences between participants. Second, the misreporting of food consumption and the missing of packaging materials information represent a potential limitation, influencing the observed relationship between food contaminants exposure and health outcomes. To overcome this limitation, several days of dietary information were used in all papers. Even though, considering that usually under-report is more frequent than over-report (34) and given the statistically significant results that were found in our sample, we can hypothesise that the effects observed in young consumers of the general population would probably be even more pronounced. Third these results may not accurately reflect current levels, as the occurrence database was compiled before 2015. Furthermore, considering that in 2017, the European Commission (EC) introduced a Regulation establishing mitigation measures and benchmark levels to reduce acrylamide occurrence in foods, we may hypothesise that this exposure started to decrease after this time mark. Nevertheless, without studies assessing this Regulation's effectiveness and re-evaluating the risks to public health related to the presence of acrylamide in foodstuffs, the extent to which industries and producers have responded to this Regulation may be questionable. Fourth, the application of an upper-bound scenario approach may overestimate the exposure to food contaminants. However, we decided to follow a more conservative approach to assess the worst-case scenario for consumers. Fifth, although our papers utilised a longitudinal design, its observational nature prevents direct causal inferences. Even though the exposure of interest was measured early in life, prior to the outcomes, enabling us to account for the temporality of the associations.

The main strength of this thesis is the inclusion of data collected from a prospective population-based birth cohort and from a representative sample of the Portuguese population. These studies supported a robust and comprehensive database, enabling detailed characterisation of individuals and their behaviours. First, considering the information from a cross-sectional sample of a prospective population-based birth cohort and from a national survey, it was possible to define exposure assessment methodologies and perform an overall descriptive analysis. Then, to address the existing knowledge gap, data from the prospective GXXI birth cohort was used to assess the longitudinal associations

between food contaminants exposure and health outcomes from childhood to adolescence. For both samples, dietary information was collected through a standardised European methodology. Different methodological approaches were used for the exposure assessment, which represents another important strength. A probabilistic method was applied to estimate acrylamide dietary exposure, considering the uncertainties of intra- and inter-variability, whereas direct and indirect methods were used to estimate BPA exposure. These approaches were rarely applied in previous studies, especially considering that BPA urinary concentration was measured in 24-hour urine samples instead of spot samples. With the application of the random forest method developed in the scope of this thesis, it was possible to estimate BPA daily exposure for the remaining sample/population, even with a relatively small number of urine samples. Finally, the use of combined exposures rather than only single exposures to test the association with impaired pubertal development.

3.3. Public health implications and future research

With the results of the present thesis, we try to fill the knowledge gap regarding the effect of food contaminants exposure on health outcomes from childhood to adolescence. We expect that our findings will contribute to reducing the uncertainties previously identified by other authors and by different food safety authorities.

Firstly, data from IAN-AF show that exposure to acrylamide in more than 99.9% of Portuguese children and adolescents presented a significant public health concern regarding neoplastic effects. In children and adolescents from GXXI, BPA exposure largely exceeded the TDI of 0.2 ng/kg bw/day proposed by ESFA in 2023.

Secondly, although the mechanisms linking exposure to food contaminants with adverse health outcomes are not completely understood, and the time required for the disease's manifestation usually prevents us from detecting these effects at young ages, our results show several adverse associations that cannot be ignored. Furthermore, some of the observed associations were longitudinal

proving that early exposures influence the development of diseases in future stages of life.

For acrylamide exposure, the results may appear somewhat contradictory across the various cardiometabolic outcomes. On the one hand, positive longitudinal associations were found between acrylamide exposure with higher blood glucose levels, blood insulin, insulin resistance, waist circumference, and fat mass percentage. On the other hand, negative longitudinal associations were found for cholesterol, blood pressure, and body mass index z-score. According to the mode of action tested in animal models, it is plausible that acrylamide may act as an endocrine disruptor, impairing the balance of cardiometabolic biomarkers. However, studies performed in humans, such as the present one, failed to find a clear effect of this food contaminant on metabolic health. This could be attributed to the fact that dietary acrylamide exposure is integrated into our typical food patterns, and the food sources containing this contaminant also provide other compounds related to health benefits. And, effectively, when we look at the results from the paper I, the main contributor to acrylamide exposure in Portuguese children and adolescents was “bread and rusk”, an important fibre source (42). According to a previous study performed in GXXI, fibre intake was inversely associated with adiposity measures, namely z-BMI (167), which corroborates our hypothesis regarding the effect of a combination of acrylamide and food compounds.

A clearer effect was found regarding BPA exposure. From 4 to 13 years of age, higher exposure to this food contaminant was longitudinally associated with higher blood insulin levels, insulin resistance, fat mass percentage, waist circumference, and body mass index z-score. Thus, our study brought new insights showing that a higher early BPA exposure in humans can impair metabolic outcomes later in life.

Lastly, combining both food contaminants as a representation of real-world exposure, we concluded that acrylamide and BPA impaired the timing of puberty onset, especially at 10 and 13 years of age. Although they do not share the same sources, given the dietary patterns of each country and ours in particular, combined exposure to these two contaminants is expected by considering the food consumption scenario holistically (for example, consumption of tuna

sandwiches, hot dogs or other types of sandwiches; canned beverages paired with other acrylamide-rich foods, such as French fries; canned pulses and sausages as the principal protein source of the meals, etc).

The simplistic idea that an energy-imbalanced diet is the main cause of obesity, cardiometabolic, and endocrine disorders has been widely refuted in the scientific community (168). Foods and beverages are much more than just energy and nutrients, and their combination in a dietary pattern is determinant for exogenous hazardous exposure, especially in critical time windows such as childhood and adolescence. Several epidemiological and experimental animal studies concluded that early exposures influenced the life-long susceptibility of metabolic disorders in adulthood, regardless of individuals' nutritional status (168).

According to the WHO Global Health Observatory data repository (169), 31.6% of children aged 5 to 9 years and 26.1% of adolescents aged 10 to 19 years worldwide are affected by overweight or obesity. In Europe, this prevalence increases to 35.5% in children and 29.2% in adolescents. Globally, in 2019, almost 18 million deaths were attributed to cardiovascular diseases and 2 million to diabetes mellitus. The proportion of premature deaths due to non-communicable diseases in 2019 was 41.8% in the globe and 29.8% in Europe region. In Portugal, the total economic burden of overweight and obesity amounted to 1,147 million euros, with the majority of costs attributed to obesity-related diseases and only 1% associated with the direct treatment of obesity itself. Cardiovascular diseases, along with type II diabetes, were the largest contributors to this burden, accounting for over 83% of the total economic impact (170). Nevertheless, in addition to the direct healthcare costs, non-communicable diseases in adults incur significant indirect costs, including reduced workplace productivity, increased workers' compensation claims, and lower earnings (69). Besides, hormonal disruption and impaired pubertal onset also contribute to an increase in the disease burden by elevating the risk of arterial hypertension, diabetes, obesity, infertility, poor self-esteem, and psychosocial distress later in life (171).

Thus, it becomes clear that adverse health outcomes have a relevant implication not only for the individual but also for society and for the economy (69), especially when they are initiated at younger ages. In this line, the EC implemented food

policies to protect consumers from exposure to ubiquitous food contaminants such as acrylamide and bisphenol A. Moreover, based on the most recent evidence, different public health agencies continuously monitor human exposure to food contaminants, update the safety levels and provide this information to the general population (86,172). However, the effectiveness of these measures has not yet been evaluated.

In fact, the available data on acrylamide occurrence in food after the adoption of Regulation No 2017/2158 (48) is very limited, and the EC adopted, in 2019, the Recommendation 2019/1888 setting a new non-exhaustive list of food products that must be monitored by the authorities of the Member States (173). This list includes potato products, bakery products, and cereal products, among other foodstuffs such as vegetable crisps/fries, roasted nuts, roasted oilseeds, cocoa products, caramels and nougats, and aimed to enable the quick identification of the risk to adopt new strategies to prevent or reduce the exposure to this food contaminant. However, the methods used to measure acrylamide concentration in foodstuffs are expensive and time-consuming (86), which may hinder the complete implementation of these documents by the Member States. Data from the Human BioMonitoring for the European Union study (HBM4EU), including samples of children, adolescents and adults, show a significant increase in exposure levels of acrylamide until 2017, with Portugal and Italy being the countries with the highest mean exposure values (46). After 2017, the authors found a significant change in the time-dependent slope of acrylamide exposure, which may indicate the beginning of a reduction in exposure to this food contaminant as a result of the EC measures (46). Nevertheless, due to the limited available data considering the short time since implementation, these results should be interpreted with caution, as it is not yet possible to draw definitive conclusions about the efficiency and effectiveness of such measures. Indeed, according to Portuguese data from 2018, a large proportion of bread samples presented an acrylamide concentration above the indicative values established by the EC (174), a trend also observed in other countries and across a wide range of food products, for example, bakery products, biscuits and potato products (175–178).

Concerning BPA exposure from biomonitoring data, a Swedish study found significantly decreasing temporal trends between 2009 and 2014 (179), which may be attributed to the proactive measures taken by food industries to eliminate this contaminant from their FCM, coupled with the implementation of food safety policies (54,63,64). The same decreasing temporal trend was also found in different worldwide studies, namely for children and adolescents from NHANES 2005-2016 (66) and Japanese school children between 2012 and 2017 (65). Nonetheless, the occurrence of BPA in food commodities marketed in Europe slightly lowered compared to the past (180), a result also found in different Canadian canned foods (181). As for acrylamide, there is a lack of evidence assessing the effectiveness of these measures, considering that the obligation to monitor BPA levels in foods and beverages and the transmission of these data to international food safety authorities, such as EFSA, remains in the Member States. Furthermore, the current exposure to BPA remains several times above the updated safety values proposed by EFSA in 2023, which represents an urgent public health concern that needs to be addressed. This issue becomes particularly important if we consider that, in recent years, the efforts of food industries have not been limited to reducing BPA in FCM, but also to replacing it with analogues (180), whose effects in humans were poorly studied but are hypothesised to be similar to those resulting from BPA exposure (62,179,182,183).

Considering all this knowledge, EC approved a new Regulation 2024/3190 very recently, which is expected to be in force at the end of January 2025 (67). This Regulation prohibits the use of BPA in FCM and related articles. Other bisphenols and bisphenol derivatives are also banned from being used in the BPA replacement until EFSA performs an updated risk assessment. However, this Regulation represents a substantial challenge for FCM industries, since the substitution of BPA is quite complex. BPA has been a key component in the production of FCM and articles for several decades, serving a wide range of applications that remain prevalent across the European Union. Moreover, with the established transition period, we expect that the occurrence of BPA in food will persist in the near future, making it necessary to conduct a thorough

assessment of the effectiveness of this measure’s implementation after 18 months while reassessing consumers' dietary exposure.

In light of the current evidence found in the present thesis and considering the uncertainties pointed out in the discussion section, future perspectives should be focused on reducing exposure to food contaminants, namely acrylamide and bisphenol A, with intervention programs and/or communication risk actions targeted to children, adolescents and their main caregivers, as well as food industries and local producers.

Despite the widespread reliance and convenience of ready-to-eat and processed food commodities, a raised awareness is needed to sensitise the general consumer about the presence of food contaminants. In the past few years, different authors concluded that the consumption of ultra-processed foods was an important source of exposure to acrylamide (184), whereas the consumption of minimally processed foods was associated with lower exposure to bisphenol A (185). Thus, the key message to consumers must be to advise them to make better food choices, such as selecting fresh and unpackaged foodstuffs or prioritising foods packaged in glass, reading the labels, and following the recommended storage conditions.

Individual dietary pattern is a modifiable factor that critically accelerates or delays the development of adverse health outcomes. Without changing our culture, diet safety and quality may be improved to reduce exposure to food contaminants. At home and back to our origins, people should use mild cooking temperatures, adopting boiling, stewing, casseroles, and braising as cooking methods over roasting and frying. Burning surfaces must be avoided by the consumers, and some moisture must be added to oven dishes.

Food industries should continue to make efforts to reduce the occurrence of acrylamide and bisphenol A in food and beverages by following the EC Regulations, protecting consumers from the rise of adverse health consequences later in life. The European example may eventually be followed by other countries worldwide, as they observe the recently implemented mitigation measures and policies and adopt them within their communities, safeguarding the health of their consumers.

Finally, future research evaluating the effectiveness of mitigation measures and food policies is needed for acrylamide and bisphenol A. It is essential to monitor the occurrence of these contaminants in food products available on the market, updating the exposure assessment and the risk characterisation to consumers. Additionally, the following risk assessment studies, estimating other food contaminants, should follow probabilistic models or combine data from food contaminants biomarkers and self-reported dietary information. Given the diet complexity and the real-world hazardous exposure, our findings suggest that toxicological studies with longitudinal designs, considering mixtures of food contaminants rather than single exposures, are needed in the near future. The black box of foodomics and the questions surrounding the effects of epigenetic modifications represent a significant unexplored frontier, offering vast opportunities for those interested in nutritional epidemiology.

The simplistic perspective with which we interpret the expression of Jean Brillat-Savarin, "*Dites-moi ce que vous mangez et je vais vous dire ce que vous êtes",* may be blurred in certain ways. In fact, the role of diet in human health extends far beyond the apport of energy and nutrients. Only a small part of the food matrix compounds was already discovered and described, and we are not sure about the interactions of the different constituents from a dietary pattern. But perhaps this expression is not so far from the truth after all. Indeed, humans are complex organisms integrated into an equally complex and dynamic system that involves different dimensions. The role of risk factors in the manifestation of disease is not so straightforward and is strongly influenced by other aspects, such as genetic, psychological, environmental, and social factors. Therefore, perhaps we truly are a reflection of what we eat: complex and not simplistic individuals, much like our diet, which is also complex and far from simplistic.

CONCLUDING REMARKS

The present thesis found an association between food contaminants exposure, namely acrylamide and bisphenol A, and the development of adverse metabolic outcomes from childhood to adolescence.

Different methodological approaches were used to estimate the exposure to food contaminants. For acrylamide, a probabilistic approach considering self-reported dietary information was used. For bisphenol A, three methodological approaches were tested. Random forest, combining self-reported dietary data and urinary metabolites concentration, was the best methodology to estimate the daily exposure to this food contaminant. Additionally, we showed that even with a relatively small number of 24-hour urine samples, it is possible to predict the daily exposure to BPA for the remaining sample or population by applying the random forest methodology, presenting a relevant insight from this thesis.

Regarding risk assessment, the current exposure to acrylamide and bisphenol A in children and adolescents indicated a public health concern since it largely exceeds the values or the margins considered safe.

Positive longitudinal associations were found between acrylamide exposure and higher blood glucose levels, blood insulin, insulin resistance, waist circumference and fat mass percentage. In the opposite direction, a negative longitudinal association was found for cholesterol, blood pressure and body mass index z-score.

From 4 to 13 years of age, a longitudinal positive association was found between BPA exposure and higher blood insulin levels, insulin resistance, fat mass percentage, waist circumference, and body mass index z-score.

Higher exposure to acrylamide at 7, 10 and 13 years of age was negatively associated with pubertal development at 10 and 13 years in girls. A higher exposure to acrylamide at 13 years and a higher exposure to bisphenol A at 10 and 13 years were positively associated with precious pubertal development in boys. The combined exposure effect of acrylamide and bisphenol A did not significantly change the found associations.

Future studies are needed to evaluate the effectiveness of mitigation measures and assess the exposure to other food contaminants. From the public health perspective, combined efforts should be taken to reduce the occurrence of food contaminants in food products and to change the dietary patterns of consumers, especially for the major contributors and mainly targetted children, adolescents, and their main caregivers.

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REFERENCES

1. Willett W. Nutritional Epidemiology. Oxford University Press; 2012. Available from: https://doi.org/10.1093/acprof:oso/9780199754038.001.0001

2. World Health Organization. Noncommunicable diseases progress monitor 2022. 2022. Available from: https://apps.who.int/iris/bitstream/handle/10665/353048/9789240047761-eng.pdf

3. Buyukdere Y, Akyol A. From a toxin to an obesogen: a review of potential obesogenic roles of acrylamide with a mechanistic approach. Nutr Rev. 2024;82(1):128–42. Available from: https://www.scopus.com/inward/record.uri?eid=2-s2.0-85179836483&doi=10.1093%2Fnutrit%2Fnuad041&partnerID=40&md5=e41c905c04e38cf7a83d99326975b62c

4. Rather IA, Koh WY, Paek WK, Lim J. The sources of chemical contaminants in food and their health implications. Front Pharmacol. 2017;8(NOV).

5. Foster R, Lunn J. 40th Anniversary Briefing Paper: Food availability and our changing diet. Nutr Bull. 2007;32(3):187–249. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1467-3010.2007.00648.x

6. Tucker GS, Featherstone S. Essentials of Thermal Processing. Wiley; 2011. (Food science and technology). Available from: https://books.google.pt/books?id=-ioIGmVPvZwC

7. Mogol BA, Gökmen V. Thermal process contaminants: acrylamide, chloropropanols and furan. Curr Opin Food Sci. 2016;7:86–92. Available from: https://www.sciencedirect.com/science/article/pii/S2214799316000072

8. Mottram DS, Low MY, Elmore JS. The Maillard reaction and its role in the formation of acrylamide and other potentially hazardous compounds in foods. In: Skog K, Alexander J, editors. Acrylamide and Other Hazardous Compounds in Heat-Treated Foods. Cambridge: Woodhead Publishing; 2006. p. 3–22. (Woodhead Publishing Series in Food Science, Technology

and Nutrition). Available from: https://www.sciencedirect.com/science/article/pii/B978184569011350001X

9. De Long NE, Holloway AC. Early-life chemical exposures and risk of metabolic syndrome. Diabetes Metab Syndr Obes. 2017;10:101–9.

10. Murkovic M, Pedreschi F. Potentially Toxic Food Components Formed by Excessive Heat Processing. In: Uribarri J, editor. Dietary AGEs and Their Role in Health and Disease. 1st ed. Boca Raton, Florida: CRC Press; 2017. p. 87–101.

11. Koszucka A, Nowak A. Thermal processing food-related toxicants: a review. Crit Rev Food Sci Nutr. 2019;59(22):3579–96. Available from: https://doi.org/10.1080/10408398.2018.1500440

12. World Health Organization. Health Implications of Acrylamide in Food. World Health Organization. Geneva, Switzerland; 2002.

13. Codex Alimentarius Commission. Prevention and Reduction of Food and Feed Contamination. 1st ed. Rome, Italy; 2012. Available from: https://openknowledge.fao.org/server/api/core/bitstreams/1798500a-c71a-43a4-8010-a8c465e9a3e9/content

14. EFSA CONTAM Panel. Scientific Opinion on acrylamide in food. EFSA Journal. 2015;13(6).

15. EFSA CONTAM Panel. Risks for public health related to the presence of furan and methylfurans in food. EFSA Journal. 2017 Oct 1;15(10):e05005. Available from: https://doi.org/10.2903/j.efsa.2017.5005

16. Guillard V, Gaucel S, Fornaciari C, Angellier-Coussy H, Buche P, Gontard N. The Next Generation of Sustainable Food Packaging to Preserve Our Environment in a Circular Economy Context. Front Nutr. 2018;5(121). Available from: https://www.frontiersin.org/article/10.3389/fnut.2018.00121

17. Poças MFF, Oliveira JC, Pinto HJ, Zacarias ME, Hogg T. New paradigm for patterns of home packaged food intake. British Food Journal.

2010;112(5):500–10. Available from: https://www.emeraldinsight.com/doi/abs/10.1108/00070701011043754

18. Costa SA, Vilela S, Correia D, Severo M, Lopes C, Torres D. Consumption of packaged foods by the Portuguese population: type of materials and its associated factors. British Food Journal. 2021;123(2):833–46.

19. Severin I, Souton E, Dahbi L, Chagnon MC. Use of bioassays to assess hazard of food contact material extracts: State of the art. Food and Chemical Toxicology. 2017;105:429–47. Available from: https://www.scopus.com/inward/record.uri?eid=2-s2.0-85019138979&doi=10.1016%2Fj.fct.2017.04.046&partnerID=40&md5=68fa6277d77217f9c5c07ac43a67a219

20. Geueke B, Groh K, Muncke J. Food packaging in the circular economy: Overview of chemical safety aspects for commonly used materials. J Clean Prod. 2018;193:491–505. Available from: http://www.sciencedirect.com/science/article/pii/S0959652618313325

21. Rocha J, Mendes AP. Materiais em Contacto com os Alimentos – Plástico na Alimentação: Uma Ameaça? Acta Portuguesa de Nutrição. 2019;17:28–33.

22. de Fátima Poças M, Hogg T. Exposure assessment of chemicals from packaging materials in foods: a review. Vol. 18, Trends in Food Science and Technology. 2007. p. 219–30.

23. United Nations. Universal Declaration of Human Rights. Paris; 1948.

24. European Parliament and Council of the European Union. Regulation (EC) Nº 178/2002 of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Communities. 2002;L31:1–24. Available from: http://data.europa.eu/eli/reg/2002/178/oj

25. Food and Agriculture Organization of the United Nations, World Health Organization. Codex Alimentarius Commission Procedural Manual. 1st ed. Rome; 1968. Available from:

https://openknowledge.fao.org/items/4422c660-0195-40a8-8e79-986e96e233c2

26. Food and Agriculture Organization of the United Nations, World Health Organization. Codex Alimentarius Commission Procedural Manual. 28th ed. Rome; 2023. Available from: https://doi.org/10.4060/ cc5042en

27. European Food Safety Authority Scientific Committee, More SJ, Bampidis V, Benford D, Bragard C, Halldorsson TI, et al. Guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal. 2022 Oct 1;20(10):e07584. Available from: https://doi.org/10.2903/j.efsa.2022.7584

28. European Food Safety Authority. Guidance of the Scientific Committee on Use of the benchmark dose approach in risk assessment. EFSA Journal. 2009 Jun 1;7(6):1150. Available from: https://doi.org/10.2903/j.efsa.2009.1150

29. European Food Safety Authority. Opinion of the Scientific Committee on a request from EFSA related to A Harmonised Approach for Risk Assessment of Substances Which are both Genotoxic and Carcinogenic. EFSA Journal. 2005 Oct 1;3(10):282. Available from: https://doi.org/10.2903/j.efsa.2005.282

30. Lambe J. The use of food consumption data in assessments of exposure to food chemicals including the application of probabilistic modelling. Proc Nutr Soc. 2002 Feb;61(1):11–8.

31. Brouwer-Brolsma EM, Brennan L, Drevon CA, Van Kranen H, Manach C, Dragsted LO, et al. Combining traditional dietary assessment methods with novel metabolomics techniques: Present efforts by the Food Biomarker Alliance. Proceedings of the Nutrition Society. 2017 Nov 1;76(4):619–27.

32. Food and Agriculture Organization of the United Nations. Dietary Assessment: A resource guide to method selection and application in low resource settings. Vitamin B-6 Metabolism in Pregnancy, Lactation, and Infancy. Rome; 2018. Available from: https://openknowledge.fao.org/server/api/core/bitstreams/3dc75cfc-9128-4f29-9d76-8d1f792078f0/content

33. Bingham SA, Bingham SA, Bingham AM, Bingham AM. The dietary assessment of individual: methods, accuracy, new techniques and recommendations. In 1987. Available from: https://api.semanticscholar.org/CorpusID:79511108

34. Magalhães V, Severo M, Torres D, Ramos E, Lopes C. Characterizing energy intake misreporting and its effects on intake estimations, in the Portuguese adult population. Public Health Nutr. 2020;23(6):1031–40.

35. Thompson FE, Subar AF. Dietary assessment methodology. In: AM Coulston, CJ Boushey & MG Ferruzzi, eds Nutrition in the prevention and treatment of disease. 3rd ed. London: Academic Press; 2013.

36. International Life Sciences Institute. Report series - Exposure from food contact materials - Summary report of a workshop held in October 2001. Ispra, Italy; 2002. Available from: https://ilsi.eu/wp-content/uploads/sites/3/2016/06/R2002Exp_Food.pdf

37. European Chemicals Agency. Guidance on information requirements and chemical safety assessment. 2008. Available from: https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment

38. Yuan J, Che S, Zhang L, Li X, Yang J, Sun X, et al. Assessing the combinatorial cytotoxicity of the exogenous contamination with BDE-209, bisphenol A, and acrylamide via high-content analysis. Chemosphere. 2021;284(235):131346. Available from: https://doi.org/10.1016/j.chemosphere.2021.131346

39. Barabási AL, Menichetti G, Loscalzo J. The unmapped chemical complexity of our diet. Nat Food. 2020;1(1):33–7.

40. Pinho I, Rodrigues S, Franchini B, Graça P. Padrão Alimentar Mediterrânico: Promotor de Saúde. Programa Nacional para a Promoção da Alimentação Saudável - Direção-Geral da Saúde, editor. Lisboa, Portugal; 2016. 40 p. Available from: https://alimentacaosaudavel.dgs.pt/activeapp2020/wp-

content/uploads/2020/01/Padrão-Alimentar-Mediterrânico-Promotor-de-Saúde-1.pdf

41. Partidário, A. et al. A Dieta Mediterrânica Em Portugal: Cultura, Alimentação e Saúde. Universidade do Algarve, editor. Faro; 2014.

42. Lopes C, Torres D, Oliveira A, Severo M, Alarcão V, Guiomar S, et al. Inquérito Alimentar Nacional e de Atividade Física, IAN-AF 2015-2016: Relatório de resultados. Universidade do Porto, editor. Universidade do Porto. 2017. Available from: www.ian-af.up.pt

43. Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J Agric Food Chem. 2002 Aug;50(17):4998–5006.

44. Turkington C, Mitchell D. The encyclopedia of poisons and atidots. Third Edit. New York: Estate of Carol Turkington; 2009.

45. Freisling H, Moskal A, Ferrari P, Nicolas G, Knaze V, Clavel-Chapelon F, et al. Dietary acrylamide intake of adults in the European Prospective Investigation into Cancer and Nutrition differs greatly according to geographical region. Eur J Nutr. 2013;52(4):1369–80.

46. Poteser M, Laguzzi F, Schettgen T, Vogel N, Weber T, Zimmermann P, et al. Time Trends of Acrylamide Exposure in Europe: Combined Analysis of Published Reports and Current HBM4EU Studies. Toxics. 2022 Aug;10(8).

47. International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Industrial Chemicals. Vol. 60. Lyon; 1994.

48. European Commission. Commission Regulation (EU) 2017/2158: establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. Official Journal of the European Union. 2017;L 304(315):24–44. Available from: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R2158&from=EN

49. Rifai L, Saleh FA. A Review on Acrylamide in Food: Occurrence, Toxicity, and Mitigation Strategies. Int J Toxicol. 2020;39(2):93–102.

50. Govindaraju I, Sana M, Chakraborty I, Rahman MH, Biswas R, Mazumder N. Dietary Acrylamide: A Detailed Review on Formation, Detection, Mitigation, and Its Health Impacts. Foods. 2024 Feb;13(4).

51. European Commission. White Paper on Food Safety. 2000; Available from: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:51999DC0719

52. European Commission. Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. Official Journal of the European Union. 2004; Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32004R1935

53. European Commission. Commission Regulation (EC) No 2023/2006 of 22 December 2006 on good manufacturing practice for materials and articles intended to come into contact with food. Official Journal of the European Union. 2006; Available from: https://eur-lex.europa.eu/eli/reg/2006/2023/2008-04-17

54. European Commission. Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. Official Journal of the European Union. 2011; Available from: https://eur-lex.europa.eu/eli/reg/2011/10/oj

55. Kang JH, Kondo F, Katayama Y. Human exposure to bisphenol A. Toxicology. 2006;226(2):79–89. Available from: https://www.sciencedirect.com/science/article/pii/S0300483X06004057

56. European Food Safety Authority. Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA Journal. 2023 Apr 1;21(4):e06857. Available from: https://doi.org/10.2903/j.efsa.2023.6857

57. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons W V. Human exposure to bisphenol A (BPA). Reproductive Toxicology. 2007;24(2):139–77.

58. Noureddine El Moussawi S, Cladière M, Chébib H, Ouaini R, Camel V. Empirical models to predict the effect of sterilisation and storage on bisphenols migration from metallic can coatings into food simulants. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2019 Dec;36(12):1937–49.

59. Noureddine El Moussawi S, Ouaini R, Matta J, Chébib H, Cladière M, Camel V. Simultaneous migration of bisphenol compounds and trace metals in canned vegetable food. Food Chem. 2019;288:228–38. Available from: https://www.sciencedirect.com/science/article/pii/S0308814619304480

60. Martínez MA, Rovira J, Prasad Sharma R, Nadal M, Schuhmacher M, Kumar V. Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: A Catalonia (Spain) case study. Environ Res. 2018;166:25–34. Available from: https://www.sciencedirect.com/science/article/pii/S0013935118302470

61. Chang WH, Liu SC, Chen HL, Lee CC. Dietary intake of 4-nonylphenol and bisphenol A in Taiwanese population: Integrated risk assessment based on probabilistic and sensitive approach. Environmental Pollution. 2019 Jan 1;244:143–52.

62. Liu J, Wattar N, Field CJ, Dinu I, Dewey D, Martin JW. Exposure and dietary sources of bisphenol A (BPA) and BPA-alternatives among mothers in the APrON cohort study. Environ Int. 2018;119(March):319–26. Available from: https://doi.org/10.1016/j.envint.2018.07.001

63. European Commission. Commission Implementing Regulation (EU) No 321/2011 of 1 April 2011 amending Regulation (EU) No 10/2011 as regards the restriction of use of Bisphenol A in plastic infant feeding bottles. Official Journal of the European Union. 2011;L 87:1–2. Available from: http://data.europa.eu/eli/reg_impl/2011/321/oj

64. European Commission. Commission Regulation (EU) No 2018/213 of 12 February 2018 on the use of bisphenol A in varnishes and coatings intended to come into contact with food and amending Regulation (EU) No

10/2011 as regards the use of that substance in plastic food contact mat. Official Journal of the European Union. 2018;L 41:6–12. Available from: http://data.europa.eu/eli/reg/2018/213/oj

65. Gys C, Ait Y, Araki A, Bastiaensen M, Caballero-casero N, Kishi R, et al. Biomonitoring and temporal trends of bisphenols exposure in Japanese school children. Environ Res. 2020;191(August):110172. Available from: https://doi.org/10.1016/j.envres.2020.110172

66. Li R, Zhan W, Ren J, Zhang F, Huang X, Ma Y. Temporal trends in risk of bisphenol A, benzophenone-3 and triclosan exposure among U.S. children and adolescents aged 6–19 years: Findings from the National Health and Nutrition Examination Survey 2005–2016. Environ Res. 2023 Jan 1;216.

67. European Commission. Commission Regulation (EU) 2024/3190 of 19 December 2024 on the use of bisphenol A (BPA) and other bisphenols and bisphenol derivatives with harmonised classification for specific hazardous properties in certain materials and articles intended to come int. Official Journal of the European Union. 2024; Available from: http://data.europa.eu/eli/reg/2024/3190/oj

68. Popkin BM, Adair LS, Ng SW. Global nutrition transition and the pandemic of obesity in developing countries. Nutr Rev. 2012 Jan 1;70(1):3–21. Available from: https://doi.org/10.1111/j.1753-4887.2011.00456.x

69. Zobel EH, Hansen TW, Rossing P, von Scholten BJ. Global Changes in Food Supply and the Obesity Epidemic. Curr Obes Rep. 2016;5(4):449–55. Available from: https://doi.org/10.1007/s13679-016-0233-8

70. Ludwig DS. Technology, diet, and the burden of chronic disease. JAMA. 2011;305(13):1352–3.

71. Popkin BM. Contemporary nutritional transition: determinants of diet and its impact on body composition. Proc Nutr Soc. 2011 Feb;70(1):82–91.

72. Chong MFF. Dietary trajectories through the life course: opportunities and challenges. British Journal of Nutrition. 2022/04/27. 2022;128(1):154–9. Available from:

https://www.cambridge.org/core/product/1EE5008166B0647488E35B1FF508DAB5

73. Patton GC, Neufeld LM, Dogra S, Frongillo EA, Hargreaves D, He S, et al. Nourishing our future: the Lancet Series on adolescent nutrition. The Lancet. 2022;399(10320):123–5. Available from: http://dx.doi.org/10.1016/S0140-6736(21)02140-1

74. World Health Organization Regional Office for Europe. Food and nutrition policy for schools : a tool for the development of school nutrition programmes in the European Region. Copenhagen : WHO Regional Office for Europe; 2006. Available from: https://iris.who.int/handle/10665/107797

75. Huybrechts I, Sioen I, Boon PE, Ruprich J, Lafay L, Turrini A, et al. Dietary exposure assessments for children in Europe (the EXPOCHI project): Rationale, methods and design. Archives of Public Health. 2011;69(1):1–12. Available from: http://www.archpublichealth.com/content/69/1/4

76. Mihalache OA, Dall'Asta C. The burden of disease due to dietary exposure to acrylamide in Italy: A risk assessment-based approach. Food and Chemical Toxicology. 2024;188:114699. Available from: https://www.sciencedirect.com/science/article/pii/S0278691524002655

77. Kroes R, Mu D, Massey R, Mayer S, Urieta I, Verger P, et al. Assessment of intake from the diet. 2002;40:327–85.

78. Grados L, Pérot M, Barbezier N, Delayre-Orthez C, Bach V, Fumery M, et al. How advanced are we on the consequences of oral exposure to food contaminants on the occurrence of chronic non communicable diseases? Chemosphere. 2022;303(June).

79. Rang HP, Hill RG. Assessing drug safety. In: Hill RG, Rang HPBTDD and D (Second E, editors. Drug Discovery and Development. 2nd ed. Churchill Livingstone; 2013. p. 211–25. Available from: https://www.sciencedirect.com/science/article/pii/B9780702042997000159

80. Miller MJ, Carter DE, Sipes IG. Pharmacokinetics of acrylamide in Fisher-344 rats. Toxicol Appl Pharmacol. 1982 Mar;63(1):36–44.

81. Calleman CJ. The Metabolism and Pharmacokinetics of Acrylamide: Implications for Mechanisms of Toxicity and Human Risk Estimation. Drug Metab Rev. 1996 Jan 1;28(4):527–90. Available from: https://doi.org/10.3109/03602539608994018

82. Yener Y, Sur E, Telatar T, Oznurlu Y. The effect of acrylamide on alpha-naphthyl acetate esterase enzyme in blood circulating lymphocytes and gut associated lymphoid tissues in rats. Exp Toxicol Pathol. 2013 Jan;65(1–2):143–6.

83. Shipp A, Lawrence G, Gentry R, McDonald T, Bartow H, Bounds J, et al. Acrylamide: review of toxicity data and dose-response analyses for cancer and noncancer effects. Crit Rev Toxicol. 2006;36(6–7):481–608.

84. Park HR, Kim MS, Kim SJ, Park M, Kong KH, Kim HS, et al. Acrylamide induces cell death in neural progenitor cells and impairs hippocampal neurogenesis. Toxicol Lett. 2010 Mar;193(1):86–93.

85. LoPachin RM, Gavin T. Molecular mechanism of acrylamide neurotoxicity: lessons learned from organic chemistry. Environ Health Perspect. 2012 Dec;120(12):1650–7.

86. Sarion C, Codină GG, Dabija A. Acrylamide in Bakery Products: A Review on Health Risks, Legal Regulations and Strategies to Reduce Its Formation. Vol. 18, International Journal of Environmental Research and Public Health. 2021.

87. Hamdy SM, Bakeer HM, Eskander EF, Sayed ON. Effect of acrylamide on some hormones and endocrine tissues in male rats. Hum Exp Toxicol. 2011 Aug 30;31(5):483–91. Available from: https://doi.org/10.1177/0960327111417267

88. Sto M, Matavulj M, Markovi J. Subchronic exposure to acrylamide leads to pancreatic islet remodeling determined by alpha cell expansion and beta cell mass reduction in adult rats. Acta Histochem. 2018;120(February):228–35.

89. Amato AA, Wheeler HB, Blumberg B. Obesity and endocrine-disrupting chemicals. Endocr Connect. 2021 Feb;10(2):R87–105.

90. Wang B, Qiu W, Yang S, Cao L, Zhu C, Ma J, et al. Acrylamide exposure and oxidative dna damage, lipid peroxidation, and fasting plasma glucose alteration: Association and mediation analyses in chinese urban adults. Diabetes Care. 2020;43(7):1479–86.

91. Wan X, Zhu F, Zhuang P, Liu X, Zhang L, Jia W, et al. Associations of Hemoglobin Adducts of Acrylamide and Glycidamide with Prevalent Metabolic Syndrome in a Nationwide Population-Based Study. 2022;

92. Hung CC, Cheng YW, Chen WL, Fang WH. Negative Association between Acrylamide Exposure and Metabolic Syndrome Markers in Adult Population. Int J Environ Res Public Health. 2021 Nov;18(22).

93. Vesper HW, Slimani N, Hallmans G, Tjønneland A, Agudo A, Benetou V, et al. Cross-sectional study on acrylamide hemoglobin adducts in subpopulations from the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. J Agric Food Chem. 2008 Aug;56(15):6046–53.

94. Huang M, Zhuang P, Jiao J, Wang J, Zhang Y. Association of acrylamide hemoglobin biomarkers with obesity, abdominal obesity and overweight in general US population: NHANES 2003 – 2006. Science of the Total Environment. 2018;631–632:589–96. Available from: https://doi.org/10.1016/j.scitotenv.2018.02.338

95. Chu PL, Lin LY, Chen PC, Su TC, Lin CY. Negative association between acrylamide exposure and body composition in adults: NHANES, 2003-2004. Nutr Diabetes. 2017 Mar;7(3):e246.

96. Liu Z, Wang J, Chen S, Xu C, Zhang Y. Associations of acrylamide with non-alcoholic fatty liver disease in American adults: a nationwide cross-sectional study. Environ Health. 2021 Aug;20(1):98.

97. Lin CY, Lin YC, Kuo HK, Hwang JJ, Lin JL, Chen PC, et al. Association among acrylamide, blood insulin, and insulin resistance in adults. Diabetes Care. 2009 Dec;32(12):2206–11.

98. Cheang I, Liao S, Zhu X, Lu X, Zhu Q, Yao W, et al. Association of acrylamide hemoglobin biomarkers with serum lipid levels in general US

population: NHANES 2013-2016. Ecotoxicol Environ Saf. 2021 May;214:112111.

99. Matoso V, Bargi-Souza P, Ivanski F, Romano MA, Romano RM. Acrylamide: A review about its toxic effects in the light of Developmental Origin of Health and Disease (DOHaD) concept. Food Chem. 2019 Jun;283:422–30.

100. Gao JG, Jiang Y, Zheng JT, Nie LW. Pubertal exposure to acrylamide disrupts spermatogenesis by interfering with meiotic progression in male mice. Toxicol Lett. 2022 Apr;358:80–7.

101. Yu Y, Zhang D, Xu J, Zhang D, Yang L, Xia R, et al. Adolescence is a sensitive period for acrylamide-induced sex hormone disruption: Evidence from NHANES populations and experimental mice. Ecotoxicol Environ Saf. 2023 Jan;249:114413.

102. Nagata C, Konishi K, Wada K, Tamura T, Goto Y, Koda S, et al. Associations of Acrylamide Intake With Urinary Sex Hormone Levels Among Preschool-Age Japanese Children. Am J Epidemiol. 2018 Jan;187(1):75–81.

103. Nagata C, Wada K, Yamakawa M, Sugino M, Mori T, Ueyama J, et al. Acrylamide exposure, sex hormones, and pubertal status in Japanese adolescents. Int J Environ Health Res. 2024 Sep;1–10.

104. Chu PL, Liu HS, Wang C, Lin CY. Association between acrylamide exposure and sex hormones in males: NHANES, 2003–2004. PLoS One. 2020 Jun 18;15(6):e0234622. Available from: https://doi.org/10.1371/journal.pone.0234622

105. EU-RAR (European Union Risk Assessment Report). 4,4'-isopropylidenediphenol (Bisphenol-A). Human Health, Part 2. Publications Office of the European Union. 2008; Available from: https://publications.jrc.ec.europa.eu/repository/handle/JRC59988

106. Stahlhut RW, Welshons W V., Swan SH. Bisphenol A data in NHANES suggest longer than expected half-life, substantial nonfood exposure, or both. Environ Health Perspect. 2009;117(5):784–9.

107. Thayer KA, Doerge DR, Hunt D, Schurman SH, Twaddle NC, Churchwell MI, et al. Pharmacokinetics of bisphenol A in humans following a single oral administration. Environ Int. 2015 Oct;83:107–15.

108. Meslin M, Beausoleil C, Zeman FA, Antignac JP, Kolossa-Gehring M, Rousselle C, et al. Human Biomonitoring Guidance Values (HBM-GVs) for Bisphenol S and Assessment of the Risk Due to the Exposure to Bisphenols A and S, in Europe. 2022; Available from: https://doi.org/10.3390/toxics10050228

109. Von Goetz N, Wormuth M, Scheringer M, Hungerbühler K. Bisphenol A: How the most relevant exposure sources contribute to total consumer exposure. Risk Analysis. 2010 Mar;30(3):473–87.

110. Cousins IT, Staples CA, Klečka GM, Mackay D. A Multimedia Assessment of the Environmental Fate of Bisphenol A. Human and Ecological Risk Assessment: An International Journal. 2002 Jul 1;8(5):1107–35. Available from: https://doi.org/10.1080/1080-700291905846

111. Giulivo M, Lopez de Alda M, Capri E, Barceló D. Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. A review. Environ Res. 2016;151:251–64. Available from: https://www.scopus.com/inward/record.uri?eid=2-s2.0-84982863801&doi=10.1016%2Fj.envres.2016.07.011&partnerID=40&md5=a13440caf591f18cb10588ebe3c73b87

112. Rochester JR. Bisphenol A and human health: A review of the literature. Vol. 42, Reproductive Toxicology. 2013. p. 132–55.

113. Moon S, Hoon S, Beom C, Joo Y, Joon H, Sun D. Effects of bisphenol A on cardiovascular disease : An epidemiological study using National Health and Nutrition Examination Survey 2003 – 2016 and meta-analysis. Science of the Total Environment. 2021;763:142941. Available from: https://doi.org/10.1016/j.scitotenv.2020.142941

114. European Food Safety Authority. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA Journal. 2015;13(1).

115. Luo S, Li Y, Li Y, Zhu Q, Jiang J, Wu C, et al. Gestational and lactational exposure to low-dose bisphenol A increases Th17 cells in mice offspring. Environ Toxicol Pharmacol. 2016;47:149–58. Available from: https://www.sciencedirect.com/science/article/pii/S1382668916302472

116. Amin MM, Ebrahim K, Hashemi M, Shoshtari-Yeganeh B, Rafiei N, Mansourian M, et al. Association of exposure to Bisphenol A with obesity and cardiometabolic risk factors in children and adolescents. Int J Environ Health Res. 2019;29(1):94–106. Available from: https://doi.org/10.1080/09603123.2018.1515896

117. Wang HX, Zhou Y, Tang CX, Wu JG, Chen Y, Jiang QW. Association between bisphenol A exposure and body mass index in Chinese school children: A cross-sectional study. Environ Health. 2012;11(1):1–9.

118. Eng DS, Lee JM, Gebremariam A, Meeker JD, Peterson K, Padmanabhan V. Bisphenol A and chronic disease risk factors in US children. Pediatrics. 2013;132(3).

119. Delepierre J, Fosse-Edorh S, Fillol C, Piffaretti C. Relation of urinary bisphenol concentration and diabetes or prediabetes in French adults: A cross-sectional study. PLoS One. 2023;18(3 March):1–11.

120. Dunder L, Lejonklou MH, Lind PM, Lind L. Urinary bisphenol A and serum lipids: A meta-analysis of six NHANES examination cycles (2003-2014). J Epidemiol Community Health (1978). 2019;73(11):1012–9.

121. Braun JM, Lanphear BP, Calafat AM, Deria S, Khoury J, Howe CJ, et al. Early-life bisphenol a exposure and child body mass index: a prospective cohort study. Environ Health Perspect. 2014 Nov;122(11):1239–45.

122. Salamanca-Fernández E, Vela-Soria F, Rodríguez-Barranco M, Arrebola-Moreno A, Iribarne-Durán LM, Olea N, et al. Serum levels of non-persistent environmental pollutants and risk of incident hypertension in a sub-cohort from the EPIC study. 2021;193(110491).

123. Mouneimne Y, Nasrallah M, Khoueiry-Zgheib N, Nasreddine L, Nakhoul N, Ismail H, et al. Bisphenol A urinary level, its correlates, and association with cardiometabolic risks in Lebanese urban adults. Environ Monit Assess. 2017;189(10).

124. Ribeiro CM, Beserra BTS, Silva NG, Lima CL, Rocha PRS, Coelho MS, et al. Exposure to endocrine-disrupting chemicals and anthropometric measures of obesity: a systematic review and meta-analysis. BMJ Open. 2020 Jun;10(6):e033509.

125. Lin M hsun, Lee C ying, Chuang Y shiuan, Shih C lung. Exposure to bisphenol A associated with multiple health-related outcomes in humans : An umbrella review of systematic reviews with meta-analyses. Environ Res. 2023;237(April).

126. Bigambo FM, Sun H, Yan W, Wu D, Xia Y, Wang X, et al. Association between phenols exposure and earlier puberty in children: A systematic review and meta-analysis. Environ Res. 2020 Nov;190:110056.

127. Choe Y, Kim K nam, Jeong Y, Inhyang J, Kim B nyun, Lim Y hee, et al. Prenatal and childhood exposure to endocrine-disrupting chemicals and early thelarche in 8-year-old girls : A prospective study using Bayesian kernel regression. Environ Res. 2024;263(P1):120056. Available from: https://doi.org/10.1016/j.envres.2024.120056

128. Chen Y, Wang Y, Ding G, Tian Y, Zhou Z, Wang X, et al. Association between bisphenol a exposure and idiopathic central precocious puberty (ICPP) among school-aged girls in Shanghai, China. Environ Int. 2018;115:410–6. Available from: https://www.sciencedirect.com/science/article/pii/S0160412017316689

129. Calcaterra V, Cena H, Loperfido F, Rossi V, Grazi R, Quatrale A, et al. Evaluating Phthalates and Bisphenol in Foods: Risks for Precocious Puberty and Early-Onset Obesity. Nutrients. 2024 Aug;16(16).

130. Uldbjerg CS, Koch T, Lim YH, Gregersen LS, Olesen CS, Andersson AM, et al. Prenatal and postnatal exposures to endocrine disrupting chemicals

and timing of pubertal onset in girls and boys: a systematic review and meta-analysis. Hum Reprod Update. 2022 Aug;28(5):687–716.

131. Larsen PS, Kamper-Jørgensen M, Adamson A, Barros H, Bonde JP, Brescianini S, et al. Pregnancy and birth cohort resources in Europe: A large opportunity for aetiological child health research. Vol. 27, Paediatric and Perinatal Epidemiology. Blackwell Publishing Ltd; 2013. p. 393–414.

132. Lopes C, Torres D, Oliveira A, Severo M, Guiomar S, Alarcão V, et al. National food, nutrition, and physical activity survey of the Portuguese general population (2015-2016): Protocol for design and development. J Med Internet Res. 2018;20(2):1–11. Available from: www.ian-af.up.pt

133. World Medical Association. WMA Declaration of Helsinki: ethical principles for medical research involving human subjects. 1974;353(1):1418–9. Available from: http://www.wma.net/en/30publications/10policies/b3/index.html

134. Gibson RS. Principles of Nutritional Assessment. 2nd ed. New York: Oxford University Press; 2005.

135. WHO Multicentre Growth Reference Study Group. Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age Methods and development. Geneva; 2006.

136. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Manuel Gómez J, et al. Bioelectrical impedance analysis - part II: utilization in clinical practice. Clinical Nutrition. 2004 Dec 1;23(6):1430–53. Available from: https://doi.org/10.1016/j.clnu.2004.09.012

137. Schaefer F, Georgi M, Zieger A, Schärer K. Usefulness of bioelectric impedance and skinfold measurements in predicting fat-free mass derived from total body potassium in children. Pediatr Res. 1994 May;35(5):617–24.

138. Harrington DM, Staiano AE, Broyles ST, Gupta AK, Katzmarzyk PT. Waist circumference measurement site does not affect relationships with visceral adiposity and cardiometabolic risk factors in children. Pediatr Obes. 2013

Jun 1;8(3):199–206. Available from: https://doi.org/10.1111/j.2047-6310.2012.00106.x

139. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics. 2004 Aug;114(2 Suppl 4th Report):555–76.

140. Cunha SC, Fernandes JO. Quantification of free and total bisphenol A and bisphenol B in human urine by dispersive liquid-liquid microextraction (DLLME) and heart-cutting multidimensional gas chromatography-mass spectrometry (MD-GC/MS). Talanta. 2010;83(1):117–25. Available from: http://dx.doi.org/10.1016/j.talanta.2010.08.048

141. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969 Jun;44(235):291–303.

142. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child. 1970 Feb;45(239):13–23.

143. US Department of Agriculture Agricultural Research Service. USDA National Nutrient Database for Standard Reference: Nutrient Data Laboratory. www.ars.usda.gov. 2004.

144. European Food Safety Authority. Guidance on the EU Menu methodology. EFSA Journal. 2014;12(12).

145. Instituto Nacional de Estatistica (Portugal). The new Classification of urban areas. Deliberation of the Standing Section of Statistical Coordination of the Statistical Council 2014. Available from: www.ine.pt

146. Goios ACL, Severo M, Lloyd AJ, Magalhães VPL, Lopes C, Torres DPM. Validation of a new software eAT24 used to assess dietary intake in the adult Portuguese population. Public Health Nutr. 2020;(3):1–11.

147. European Food Safety Authority. Report on the development of a Food Classification and Description System for exposure assessment and guidance on its implementation and use. EFSA Journal. 2011;9(12):1–84.

148. Torres D, Faria N, Sousa N, Soares R, Amorim H, Guiomar S, et al. Manual Fotográfico de Quantificação de Alimentos. Universidade do Porto, 2017; 2017. Available from: www.ian-af.up.pt

149. Jeong H, Hwang S, Kwon H. Survey for acrylamide in processed foods from Korean market and individual exposure estimation using a non-parametric probabilistic model. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2020 Jun;37(6):916–30.

150. Trasande L, Shaffer RM, Sathyanarayana S. Food additives and child health. Pediatrics. 2018;142(2). Available from: https://www.scopus.com/inward/record.uri?eid=2-s2.0-85051081153&doi=10.1542%2Fpeds.2018-1410&partnerID=40&md5=75c9e708dd736de403c2f8b52d3e13f6

151. McBride DL. Safety Concerns About Food Additives and Children's Health. Journal of Pediatric Nursing: Nursing Care of Children and Families. 2019;45:76–7. Available from: https://doi.org/10.1016/j.pedn.2018.09.008

152. Sirot V, Rivière G, Leconte S, Vin K, Traore T, Jean J, et al. French infant total diet study: Dietary exposure to heat-induced compounds (acrylamide, furan and polycyclic aromatic hydrocarbons)and associated health risks. Food and Chemical Toxicology. 2019;130(May):308–16. Available from: https://doi.org/10.1016/j.fct.2019.05.009

153. Food Safety Authority of Ireland. Report on a total diet study carried out by the Food Safety Authority of Ireland in the period 2012 – 2014. 2016. Available from: www.fsai.ie

154. Gorecki S, Bemrah N, Roudot AC, Marchioni E, Le Bizec B, Faivre F, et al. Human health risks related to the consumption of foodstuffs of animal origin contaminated by bisphenol A. Food and Chemical Toxicology. 2017;110:333–9. Available from: https://www.sciencedirect.com/science/article/pii/S0278691517306439

155. Yao K, Zhang J, Yin J, Zhao Y, Shen J, Jiang H, et al. Bisphenol A and Its Analogues in Chinese Total Diets: Contaminated Levels and Risk Assessment. Oxid Med Cell Longev. 2020;2020.

156. Valer-Martinez A, Sayon-Orea C, Martínez Hernandez JA, De la Fuente-Arrillaga C, Pérez de Rojas J, Barcones F, et al. Forecasting levels of serum 25-hydroxyvitamin D based on dietary intake, lifestyle and personal determinants in a sample of Southern Europeans. British Journal of Nutrition. 2023 Apr 11 [cited 2023 Jul 5];1–9. Available from: https://www.cambridge.org/core/product/identifier/S0007114523000946/type/journal_article

157. Tao L, Tang W, Xia Z, Wu B, Liu H, Fu J, et al. Machine learning predicts the serum PFOA and PFOS levels in pregnant women: Enhancement of fatty acid status on model performance. Environ Int. 2024;190:108837. Available from: https://www.sciencedirect.com/science/article/pii/S0160412024004239

158. Yang Y, Gu Y, Zhang Y, Zhou Q, Zhang S, Wang P, et al. Spatial - temporal mapping of urine cadmium levels in China during 1980 - 2040: Dietary improvements lower exposure amid rising pollution. J Hazard Mater. 2024;473:134693. Available from: https://www.sciencedirect.com/science/article/pii/S030438942401272X

159. Khanam R, Nayak AK, Kulsum PGPS, Mandal J, Shahid M, Tripathy R, et al. Silica sources for arsenic mitigation in rice: machine learning-based predictive modeling and risk assessment. Environ Sci Pollut Res Int. 2023 Nov;30(53):113660–73.

160. Loos RJF, Yeo GSH. The genetics of obesity: from discovery to biology. Nat Rev Genet. 2022 Feb;23(2):120–33.

161. Vafeiadi M, Roumeliotaki T, Myridakis A, Chalkiadaki G, Fthenou E, Dermitzaki E, et al. Association of early life exposure to bisphenol A with obesity and cardiometabolic traits in childhood. Environ Res. 2016 Apr;146:379–87.

162. Lee HA, Kim YJ, Lee H, Gwak HS, Park EA, Cho SJ, et al. Effect of urinary bisphenolA on androgenic hormones and insulin resistance in preadolescent girls: a pilot study from the Ewha Birth & Growth Cohort. Int J Environ Res Public Health. 2013 Nov;10(11):5737–49.

163. Wang Z, Li D, Miao M, Liang H, Chen J, Zhou Z, et al. Urine bisphenol A and pubertal development in boys. Int J Hyg Environ Health. 2017;220(1):43–50. Available from: https://www.sciencedirect.com/science/article/pii/S1438463916304254

164. Durmaz E, Aşçı A, Erkekoğlu P, Akçurin S, Koçer Gümüşel B, Bircan İ. Urinary Bisphenol A Levels in Girls with Idiopathic Central Precocious Puberty. J Clin Res Pediatr Endocrinol. 2014;6(1):16–21. Available from: https://dx.doi.org/10.4274/Jcrpe.1220

165. Kasper-Sonnenberg M, Wittsiepe J, Wald K, Koch HM, Wilhelm M. Pre-pubertal exposure with phthalates and bisphenol A and pubertal development. PLoS One. 2017;12(11):e0187922.

166. Frederiksen H, Aksglaede L, Sorensen K, Nielsen O, Main KM, Skakkebaek NE, et al. Bisphenol A and other phenols in urine from Danish children and adolescents analyzed by isotope diluted TurboFlow-LC–MS/MS. Int J Hyg Environ Health. 2013;216(6):710–20. Available from: https://www.sciencedirect.com/science/article/pii/S1438463913000163

167. Marinho AR, Severo M, Vilela S, Torres D, Oliveira A, Lopes C. Association of dietary macronutrient intake with adiposity during childhood according to sex: Findings from the generation XXI birth cohort. Pediatr Obes. 2022 Sep;17(9):e12916.

168. Guzylack-Piriou L, Ménard S. Early life exposure to food contaminants and social stress as risk factor for metabolic disorders occurrence?— an overview. Biomolecules. 2021;11(5).

169. World Health Organization. The Global Health Observatory. Available from: https://www.who.int/data/gho

170. Borges M, Sampaio F, Costa J, Freitas P, Matias Dias C, Gaio V, et al. Burden of Disease and Cost of Illness of Overweight and Obesity in Portugal. Obes Facts. 2024 Nov 5;1–14. Available from: https://doi.org/10.1159/000541781

171. Abreu AP, Kaiser UB. Pubertal development and regulation. Lancet Diabetes Endocrinol. 2016 Mar;4(3):254–64.

172. Martínez-Ibarra A, Martínez-Razo LD, MacDonald-Ramos K, Morales-Pacheco M, Vázquez-Martínez ER, López-López M, et al. Multisystemic alterations in humans induced by bisphenol A and phthalates: Experimental, epidemiological and clinical studies reveal the need to change health policies. Environmental Pollution. 2021;271.

173. European Commission. Commission Recommendation (EU) 2019/1888 of 7 November 2019 on the monitoring of the presence of acrylamide in certain foods. Official Journal of the European Union. 2019;L 290(31):31–33.

174. Jesus S, Delgado I, Rego A, Brandão C, dos Santos R, Bordado J, et al. Determination of Acrylamide in Portuguese Bread by UPLC-MS/MS: Metrological and Chemometric tools. ACTA IMEKO. 2018;7:96.

175. Branciari R, Roila R, Ranucci D, Altissimi MS, Mercuri ML, Haouet NM. Estimation of acrylamide exposure in Italian schoolchildren consuming a canteen menu: health concern in three age groups. Int J Food Sci Nutr. 2020;71(1):122–31. Available from: https://doi.org/10.1080/09637486.2019.1624692

176. Esposito F, Velotto S, Rea T, Stasi T, Cirillo T. Occurrence of Acrylamide in Italian Baked Products and Dietary Exposure Assessment. Molecules. 2020;25(18):1–10.

177. Powers SJ, Mottram DS, Curtis A, Halford NG. Progress on reducing acrylamide levels in potato crisps in Europe, 2002 to 2019. Food Additives & Contaminants: Part A. 2021 May 4;38(5):782–806. Available from: https://doi.org/10.1080/19440049.2020.1871083

178. Mesías M, Morales FJ, Delgado-Andrade C. Acrylamide in biscuits commercialised in Spain: a view of the Spanish market from 2007 to 2019. Food Funct. 2019;10(10):6624–32. Available from: http://dx.doi.org/10.1039/C9FO01554J

179. Gyllenhammar I, Glynn A, Jönsson BAG, Lindh CH, Darnerud PO, Svensson K, et al. Diverging temporal trends of human exposure to

bisphenols and plastizisers, such as phthalates, caused by substitution of legacy EDCs? Environ Res. 2017 Feb;153:48–54.

180. Neri I, Russo G, Grumetto L. Bisphenol A and its analogues: from their occurrence in foodstuffs marketed in Europe to improved monitoring strategies-a review of published literature from 2018 to 2023. Arch Toxicol. 2024 Aug;98(8):2441–61.

181. Cao XL, Popovic S, Dabeka RW. Trends of bisphenol A occurrence in canned food products from 2008-2020. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2023 Jun;40(6):781–6.

182. Rochester JR, Bolden AL. Bisphenol S and F: A Systematic Review and Comparison of the Hormonal Activity of Bisphenol A Substitutes. Environ Health Perspect. 2015 Jul;123(7):643–50.

183. Gálvez-Ontiveros Y, Monteagudo C, Giles-Mancilla M, Muros JJ, Almazán V, Martínez-Burgos MA, et al. Dietary bisphenols exposure as an influencing factor of body mass index. Environ Health. 2024 Oct;23(1):93.

184. Steele EM, Buckley JP, Monteiro CA. Ultra-processed food consumption and exposure to acrylamide in a nationally representative sample of the US population aged 6 years and older. Prev Med (Baltim). 2023;174(February):107598. Available from: https://doi.org/10.1016/j.ypmed.2023.107598

185. Buckley JP, Kim H, Wong E, Rebholz CM. Ultra-processed food consumption and exposure to phthalates and bisphenols in the US National Health and Nutrition Examination Survey, 2013-2014. Environ Int. 2019 Oct;131:105057.