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Short running head: Dietary pesticide exposure and post-menopausal breast cancer risk

Abbreviations:

ADI: Acceptable Daily Intake; AhR: Aromatic hydrocarbon Receptor; ANSES: Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail ; BMR: Basal Metabolic Rate ; BC: Breast Cancer; β-HCH : β-Hexachlorocyclohexane ; BMI: Body Mass Index; CépiDC : French Centre for Epidemiology Medical Causes of Death database ; CI: Confidence Interval ; CNIL: Commission Nationale de l'Informatique et des Libertés ; CVUA: Chemisches und Veterinäruntersuchungsamt; DDE: Dichlorodiphenyldichloroethylene ; DDT: Dichlorodiphenyltrichloroethane ; DNA: Deoxyribonucleic acid ; EDI: Estimated Daily Intake; EFSA: European Food and Safety Authority; ER-/PR-: Estrogen Receptor Negative/Progesterone Receptor Negative ; FFQ: Food Frequency Questionnaire; HCB: Hexachlorobenzene ; HR: Hazard Ratio; ICD-10: International Statistical Classification of Diseases and Related Health Problems 10th Revision; IPAQ: International Physical Activity Questionnaire; IRB INSERM: Institutional Review Board of the French Institute for Health and Medical Research; NMF: Non-negative Matrix Factorization; ; OC: Organochlorine; OP: Organophosphorous;; PCTA: Pentachlorothioanisole ; PNNS: Programme National Nutrition Santé; SD: Standard Deviation; SNIIRAM: Système National d'Information Inter-Régimes de l'Assurance Maladie; sPNNS-GS2: simplified Programme National Nutrition Santé Guideline Score 2; WHO: World Health Organization.

Clinical Trial Registry: NCT03335644

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Data Share Statement:

Data of the study are protected under the protection of health data regulation set by the French National Commission for Information Technology and Liberties (Commission Nationale de l'Informatique et des Libertés, CNIL). The data are available upon reasonable request to the study's operational manager, Nathalie Pecollo (n.pecollo@eren.smbh.univ-paris13.fr), for review by the steering committee of the NutriNet-Santé study.

1 **Abstract:**

2 **Background:**

3 Some pesticides, used in large quantities in current agricultural practices all over Europe, are

4 suspected of adverse effects on human reproductive health (breast and prostate cancers),

5 through mechanisms of endocrine disruption and possible carcinogenic properties, as

6 observed in agricultural settings.

7 However, evidence on dietary pesticide exposure and breast cancer (BC) is lacking for

8 general population. We aimed to assess the associations between dietary exposure to

9 pesticides and BC risk among postmenopausal women of the NutriNet-Santé cohort.

10 **Methods**:

11 In 2014, participants completed a self-administered semi-quantitative Food Frequency

12 Questionnaire, distinguishing conventional and organic foods. Exposures to 25 active

13 substances used in EU plant protection products were estimated using a pesticide residue

14 database accounting for farming practices, from Chemisches und Veterinäruntersuchungsamt

15 Stuttgart, Germany.

16 Non-Negative Matrix Factorization (NMF), adapted for data with excess zeros, was used to

17 establish exposure profiles. The four extracted NMF components' quintiles were introduced

18 into Cox models estimating Hazard Ratio (HR) and 95% confidence interval (95% CI),

19 adjusted for known confounding factors.

20 **Results :**

21 A total of 13,149 postmenopausal women were included in the analysis(169 BC cases,

22 median follow-up=4.83 years). Negative associations between component 3, reflecting low

23 exposure to synthetic pesticides, and post-menopausal BC risk were found (HR_{Q5}=0.57;

24 95%CI(0.34;0.93), p-trend=0.006). Positive association between component 1 score (highly

25 correlated to chlorpyriphos, imazalil, malathion, thiabendazole) and postmenopausal BC risk

26   was found specifically among overweight and obese women (HR_{Q5}=4.13; 95%CI(1.50;11.44),

27   p-trend=0.006). No associations were detected for the other components.

28   **Conclusions**:

29   These associations suggest a potential role of dietary pesticide exposure on BC risk. Further

30   research is needed to investigate mechanisms and confirm these results in other populations.

31   **Keywords**: dietary exposure; pesticides; organic farming; epidemiology; breast cancer;

32   environmental health.

33

Key Messages:

- Diet is considered as the main exposure route for pesticide exposure in the general population. Dietary pesticide exposure has been rarely studied in relation with cancers.

- Non-Negative Matrix Factorization (NMF), a method adapted for data with excess zeros, was used to characterise dietary pesticide exposure profiles.

- We observed a reduction in the risk of postmenopausal breast cancer for NMF Component 3 (reflecting low exposure to several synthetic pesticides).

- A positive association between NMF component 1 score (highly correlated to chlorpyriphos, imazalil, malathion, thiabendazole) and postmenopausal breast cancer risk was observed specifically among overweight and obese women.

- For NMF Components 2 and 4, Hazard Ratios (HR) were HR $_{\text{Quintile 5 vs Quintile 1}}$ 0.96, 95% Confidence Interval (0.59; 1.56), p for trend : 0.30 and HR $_{\text{Quintile 5 vs Quintile 1}}$ 0.65, 95% Confidence Interval (0.38; 1.12), p for trend : 0.13.

34

Introduction:

36 Large quantities of plant protection products are used in current European agricultural

37 practices [1]. In particular, France has high usage of pesticides, synthetic or natural, both in

38 global tonnages (80 000 tons in 2018) and by surface area (4.45 kg/ha in 2018) [2–5].

39 Deleterious impacts of pesticides on human health have been evidenced. Various effects of

40 pesticide active substances have been documented , including genetic material alteration,

41 endocrine disrupting effects, cell apoptosis and cell signaling dysregulation, and oxidative

42 stress induction [4,6–8]. These mechanisms have been shown to be involved in carcinogenesis [9].

43 Recently, IARC classified many pesticides as "probably carcinogenic to humans" (Group 2A)

44 and "possibly carcinogenic to humans" (Group 2B) [10]. In addition, many pesticides exhibit

45 endocrine disruptors properties [7].

46 Indeed, cancer is nowadays the first or second leading cause of premature death in many

47 European countries. It is the first cause of mortality in France [11–13], breast cancer being the

48 most common and leading cause of cancer death for women in France. Associations between

49 occupational pesticide exposure in agricultural settings (involving respiratory and cutaneous

50 exposure routes) and the occurrence of some locations of cancers (myeloma, non-Hodgkin

51 lymphoma, prostate) were found in several studies[14–19]. Associations in agricultural settings

52 with other cancer locations have been reported (stomach, esophagus, liver, colorectal…), and

53 especially reproductive system cancers (prostate, breast), potentially induced by endocrine

54 disruption mechanisms [4,7,20]. Notably, associations between breast cancer risk and

55 organophosphorus pesticide exposure were found for farmer's wives in some studies [21,22].

56 However, in the general population, although food is considered as the first exposure

57 pathway, data is lacking on associations between dietary exposure to pesticides and cancer

58 [23,24]. This may be explained by three main challenges. Firstly, measuring pesticide residue

59 concentrations in food is expensive and tedious. In addition, it is difficult to measure

60 pesticides mixtures (opposed to compounds taken separately), but necessary, as it can

61 potentially lead to synergistic effects. Finally, data existing so far generally lacks of precision

62 regarding the production system (conventional vs organic), limiting proper estimation of

63 pesticide exposure.

64 Recently, a study conducted in the NutriNet-Santé cohort showed protective associations

65 between the high proportion of organic food in the diet and different types of cancers,

66 including postmenopausal breast cancer[22]. An hypothesis advanced to explain this association

67 was the potentially lower concentrations of pesticides residues in plant organic foods [25].

68 In that context, the purpose of this work was to study the associations between dietary

69 pesticide exposure profiles and breast cancer risk among postmenopausal women included in

70 the NutriNet-Santé cohort.

71

72 **Material and Methods:**

73 *Study population*

74 The NutriNet-Santé study is a web-based prospective cohort of adults launched in France in

75 May 2009 [26]. Inclusion criteria was to be aged 18 years old and over and to speak French. A

76 set of self-administered validated questionnaires [27–29] was completed online by participants at

77 baseline and repeated every year. Complementary questionnaires were regularly proposed

78 concerning dietary behaviors and specific health issue during follow-up.

79 *Dietary intake assessment*

80 A 264-item web-based self-administered semi-quantitative food frequency questionnaire

81 (Org-FFQ) distinguishing organic and conventional foods was sent to the participants

82 between June and December 2014. The Org-FFQ has been extensively described elsewhere [30].

83 Briefly, it was elaborated on the basis of an existing validated FFQ [31] to which a 5-point

84 ordinal scale was added to measure the frequency of organic food consumption. For each

85 item, participants provided their frequency of consumption and the quantity consumed

86 helping with photographs showing different portion sizes [32]. For food and beverages with an

87 existing organic version (labelled), participants answered the question "How often was the

88 product of organic origin?" by selecting 1 of the 5 following frequency modalities: never,

89 rarely, half-of-time, often, or always. The organic food consumption was then obtained by

90 attributing the respective percentages, 0, 25, 50, 75, and 100, to the modalities. Weighting and

91 sensitivity analyses for the Org-FFQ have been published elsewhere [30].

92 All food and beverage items were aggregated into 33 food groups. Nutritional values were

93 obtained from a published food composition database [33]. A global proportion (as weight) of

94 organic food in the diet was calculated as well as the proportion of organic food for each food

95 group.

96

97 *Pesticide exposure assessment*

98   Dietary pesticide exposure was estimated by combining dietary intakes of each adult with

99   pesticide residue concentration values in foods using contamination data from Chemisches

100   und Veterinäruntersuchungsamt (CVUA) Stuttgart, a European Union reference laboratory for

101   pesticides [34]. The database comprised contamination data for conventional and organic food

102   products. Twenty-five commonly used pesticides were selected among components available

103   in this database, given both their frequency of detection above the Maximum Residue Levels

104   (MRL) when sufficient data were available, and their frequency above Acceptable Daily

105   Intake (ADI) otherwise, as detailed in Baudry et al. 2019 study [35]. Pesticides commonly used

106   in organic agricultural systems (e.g. natural pyrethrins, spinosad) were also selected. These

107   criteria made it possible to take into account a broad spectrum of classes of pesticides. The

108   264 Org-FFQ items were decomposed into 442 ingredients (comprising at least 5% of at least

109   one food item). Animal-based ingredients were excluded, as CVUA encompassed plant-based

110   ingredients only. Indeed, plant-based foods have markedly more frequent and higher

111   pesticides residues levels than foods of animal origin [36]. The resulting 180 plant ingredients

112   were matched to CVUA database and then were attributed a contamination value in organic

113   and conventional farming modes (as the mean of corresponding data point). A flowchart of

114   the different steps for the decomposition and matching is shown in **Supplementary Material**

115   **1**.

116   For each ingredient/pesticide pair in conventional and organic farming, a frequency of

117   detection and a frequency of quantification were determined using the formula as follows:

118   $$Frequency\ of\ detection = 100 \times \frac{Number\ of\ analyses - Number\ of\ undetected}{Number\ of\ analyses}$$

119   $$Frequency\ of\ quantification$$

120   $$= 100 \times \frac{Number\ of\ analyses - Number\ of\ unquantified}{Number\ of\ analyses}$$

121   Treatment of data below detection limit has been extensively described elsewhere [35].

122 As food consumption data from NutriNet-Santé referred to edible foods (bone-free, peeled or

123 cooked products), edibility and cooking factors were allocated to each ingredient when

124 necessary. The same conversion factors were used for both conventional and organic

125 products. Cooking or peeling effects on pesticide residue levels were not accounted for as

126 dilution factors are not available for all food/pesticide couples [37]. For each pesticide, the

127 estimated daily intake (EDI) (in µg/kg of weight/day) under both lower- and upper-bound

128 scenarios was calculated using methods recommended by EFSA and WHO [38,39]. Lower-bound

129 (optimistic) scenario was used for this work, as more in line with available literature

130 comparing both production systems [35,40].

131 *Covariates*

132 Baseline and yearly questionnaires collected sociodemographic and lifestyle characteristics

133 such as sex, date of birth, occupation, educational level, smoking status, number of children.

134 Monthly income by household unit was obtained was obtained using both the household

135 income and composition. Anthropometric measures (height, weight), physical activity (using

136 the validated Physical Activity Questionnaire [41]) and health status (menopausal status, family

137 history of cancer, treatments) were also collected.

138 A specific questionnaire on environmental exposure collected the type of environment in

139 which participants lived: agricultural or urban area.

140 The simplified Programme National Nutrition Santé Guideline Score 2 (sPNNS-GS2), based

141 on the level of adherence to 2017 French dietary guidelines proposed by the High Council of

142 Public Health [42,43], the provegetarian score [44] and the percentage of ultra-processed foods in

143 the diet [45] were computed to be used as adjustment factors. Briefly, the sPNNS-GS2 includes

144 13 components. One point was allocated for following the guideline (and 0 otherwise), and

145 conversely for moderation components. Component with several subcomponents were

146 standardized and a penalty for overconsumption was applied. The score ranged from $-\infty$ to

147   14.25. Component, cut-off, scoring system and ponderation are presented in **Supplementary**

148   **Material 2**.

149   The provegetarian score was computed as follows [44]: 7 vegetable food groups and 5 animal

150   food groups were defined and sex-specific quintiles adjusted for total energy intake were

151   calculated. For each plant component, 1 to 5 points were allocated to quintile 1 to 5 and for

152   animal food groups the scoring was reversed. The provegetarian score was obtained by

153   summing each quintile value of vegetable food group and each reverse quintile value of

154   animal food group thus ranging from 12 (low plant food consumption) to 60 (high plant food

155   consumption).

156   Percentage of ultra-processed foods consumed was computed after classification of foods

157   using NOVA categories [45], by a committee of dietitians and researchers [46]. NOVA

158   classification is described in details in **Supplementary Material 3**. Data used to calculate the

159   proportion of ultra-processed foods in the diet were the closest to the Org-FFQ completion

160   date.

161   *Cancer cases*

162   Health events were declared by participants through a yearly health status questionnaire and a

163   dedicated web-interface at any time of the study. All medical records were collected and

164   analyzed by dedicated physicians. Physicians of participants declaring major health events

165   were contacted to collect additional information if necessary. Validation of these major health

166   events was carried out by a medical expert committee.

167   Overall, medical records were obtained for more than 90% of self-reported cancer cases.

168   Moreover, we performed a linkage between our declared health data to medico-administrative

169   registers of the national health insurance system (Système National d'Information Inter-

170   Régimes de l'Assurance Maladie [SNIIRAM] databases). Mortality data were also used from

171   the French Centre for Epidemiology Medical Causes of Death database (CépiDC). Cancer

172   cases were classified using the *International Statistical Classification of Diseases and Related*

173   *Health Problems*, *10th Revision*, *Clinical Modification* [47]. In this study, we considered all first

174   primary breast cancers (ICD-10 C50) diagnosed between baseline (i.e. the date of completion

175   of the Org-FFQ in 2014 or the menopause date, whichever occurred last) and 18 July 2019 to

176   be cases.

177   *Statistical analyses*

178   A flowchart for the study sample selection is presented in Figure 1.

179   For the present study, postmenopausal female participants who completed the Org-FFQ

180   between June and December 2014 (N = 28,445), with no missing covariates for basal

181   metabolic rate computation (N =28,137), who were not detected as under- or over-reporters

182   (N = 27,158), who were postmenopausal and free of breast cancer when they completed the

183   Org-FFQ, were selected (N=13,149).

184   Regarding under or overreporters, only participants with a plausible energy intake were

185   included in the analyses. The detection method for under and overreporters was based on the

186   comparison between energy intake and energy requirement and is extensively described in a

187   previous article by Baudry et al. [30]

188   Dietary pesticide exposure profiles were analyzed using Non-Negative Matrix factorization

189   (NMF) (detailed in **Supplementary Material 4**), specially adapted for non-negative data with

190   excess zeros, developed by Lee et al [48]. In total, four components were computed for the NMF

191   procedure using 25 selected pesticide exposure values, reflecting various pesticide exposure

192   patterns.

193   Sociodemographic and lifestyle characteristics were compared between cases and non-cases,

194   and also across NMF-extracted component quintiles using Chi², Mantel-Haenzel, Wilcoxon

195   and Kruskal-Wallis tests, as appropriate.

196 Associations between dietary pesticide exposure, using NMF components divided into

197 quintiles (first quintile used as reference) and breast cancer were assessed using Cox

198 proportional hazards regression models. Participants contributed person time until the date of

199 diagnosis of cancer, the date of last completed questionnaire, the date of death, or 18th July

200 2019, whichever occurred first.

201 NMF component scores were divided into quintiles and introduced into separate models, with

202 age as time scale, and first quintile as reference.

203 Cox models were adjusted for known confounders such as smoking status, alcohol intake,

204 educational level, physical activity (measured with International Physical Activity

205 Questionnaire), Body Mass Index (BMI), height, family history of cancer, menopausal

206 treatment and parity and overall quality of the diet (measured by the PNNS-GS2 score [43]).

207 Interactions between potential modulating factors and components were tested by introducing

208 the multiplicative interaction term into the models, namely body mass index, sPNNS-GS2

209 (overall nutritional quality of the diet) and the level of plant-based consumption (using the

210 provegetarian score). Interactions with $p<0.10$ were further investigated.

211 Schoenfeld residuals were used to test the proportional hazard assumption of the Cox model.

212 Potential nonlinear effects of continuous exposure variables were evaluated using martingale

213 residuals.

214 Tests for linear trend were performed using quintiles of the NMF components as ordinal

215 variables.

216 Sensitivity analyses were carried out. A model was performed excluding early cases (1 year

217 after baseline) and two other models were computed with additional adjustments for the level

218 of ultra-processed foods in the diet, and the provegetarian score. Two-sided tests were used..

219 Data management and statistical analyses were performed using SAS (version 9.4; SAS

220 Institute, Inc.). NMF was performed using R's NMF package [49].

221 **Results:**

222 *Characteristics of the participants*

223 Sociodemographic characteristics of the studied participants are presented in **Table 1**. A total

224 of 13,149 postmenopausal women were included in the analyses; 169 postmenopausal breast

225 cancer were diagnosed during the follow-up (mean $\pm$ SD: 4.35 $\pm$1.06 years; median: 4.83

226 years). Mean age at baseline was 60.5 years (SD=7.39). The majority of individuals had a

227 graduate educational attainment, was retired and lived in more than 200,000 inhabitant urban

228 units and were never smokers for 49% of them. One third of the sample was overweight

229 (BMI>25 kg/m²). Most frequent physical activity levels were 'high' and 'moderate'. Overall,

230 no significant differences on sociodemographic characteristics were found between cases and

231 non-cases. The nutritional characteristics of the cases and non-cases are presented

232 in**Supplementary Table S1.** Overall, no differences were observed between cases and non-

233 cases except for organic food proportion in the diet.

234 The absolute estimated dietary pesticide exposure for cases and non-cases is presented

235 inTable 2**.** Among others, the pesticide exhibiting the highest means for exposures in cases

236 and non-cases were boscalid, iprodione, spinosad, thiabendazole, and imazalil.

237 The correlations between the 4 NMF-extracted components and pesticide exposure are shown

238 **inTable 3**. Pesticides such as chlorpyriphos, imazalil, malathion, profenofos, thiabendazole

239 were highly correlated with NMF Component 1. For NMF Component 2, highly correlated

240 pesticides were azoxystrobin, boscalid, cyprodinil, difenoconazole, fenhexamid, iprodione,

241 tebuconazole, lambda cyhalothrin.

242 NMF Component 3 was characterized by low correlations with synthetic pesticides and high

243 correlation with organic pesticide spinosad. For NMF Component 4, high correlations with

244 acetamiprid, carbendazim, chlorpyriphos, cypermethrin, dimethoate/omethoate were

245 observed.

246 Each NMF Component exhibited specific correlates. For information, profiles and dietary

247   patterns are presented in **Supplementary Tables S2 to S9: Supplementary Tables S2-S3** for

248   NMF Component 1, **S4-S5** for NMF Component 2, **S6-S7** for NMF Component 3, **S8-S9** for

249   NMF Component 4. Main findings are the negative and positive linear associations between

250   proportion of organic food and NMF components 1-2 and 3 respectively.

251   The absolute estimated dietary pesticide exposures compared across components quintiles are

252   presented in **Supplementary Table S10** and **Supplementary Table S11**.

253   Correlations between dietary intakes for 33 food groups and NMF components are shown in

254   **Supplementary Table S12.**

255   *Associations between pesticide dietary exposure and breast cancer risk*

256   **Table 4** presents Hazard Ratios (HR) for the associations between NMF components and the

257   risk of postmenopausal breast cancer, with several levels of adjustments. Positive and

258   significant association was found for the fifth quintile of NMF component 1, HR=1.73,

259   95%CI(1.05;2.84). With regard to NMF component 3, participants in the fifth quintile had

260   significantly lower risks (HR=0.57, 95%CI(0.34;0.93), p trend=0.006) of postmenopausal

261   breast cancer than the first quintile (p<0.05). HR for the fifth quintiles of NMF Components 2

262   and 4 were HR 0.96, 95% CI(0.59;1.56), p-trend : 0.30 and HR 0.65, 95% CI(0.38;1.12), p-

263   trend : 0.13.

264   Further adjustments for the quality of the diet (with the sPNNS-GS2 score, Model 2), and

265   residing in an agricultural area (Model 3) did not modify the findings (**Table 4**).

266   Several interactions between NMF components and other variables were tested in the models

267   (provegetarian score, sPNNS-GS2, overweight vs non-overweight). A significant interaction

268   was found between BMI and NMF component 1 (p for interaction with BMI in 2 categories =

269   0.004) on the risk of postmenopausal breast cancer. Therefore, stratified analyses were

270   performed, with a threshold of 25 kg/m², and results are shown in **Table 5**. Associations

271   between NMF Component 1 and post-menopausal breast cancer risk were significant among

272   individuals with a BMI>25 kg/m² only, with higher risk for the fifth quintile and fourth

273   quintile compared to the first quintile, $HR_{Quintile\ 5\ vs\ Quintile\ 1}$: 4.13 (95%CI(1.50;11.44)) and

274    $HR_{Quintile\ 4\ vs\ Quntile\ 1}$: 3.02 (95%CI(1.08;8.47)), p trend=0.006 (**Table 5**).

275   *Sensitivity analyses*

276   After exclusion of cancer cases occurring less than 1 year after baseline, HR were similar but

277   the loss of statistical power did not allow to reach significance (**Table 6**).

278   Further adjustments for the percentage of ultra-processed foods, or provegetarian score, did

279   not modify the results substantially.

280 **<u>Discussion:</u>**

281 In this large population of French postmenopausal women, we found significant negative

282 associations between NMF component 3 (reflecting low exposure to several synthetic

283 pesticides) and post-menopausal breast cancer risk. When analyses were stratified on BMI

284 (threshold 25 kg/m^2), positive association between NMF Component 1 (reflecting exposure to

285 chlorpyriphos, imazalil, malathion, thiabendazole) and postmenopausal breast cancer risk was

286 found among overweight women. No significant associations were detected for the other

287 components.

288 To our knowledge, the present study is the first to investigate various pesticide exposure

289 patterns, accounting for farming practices in relation with breast cancer risk in the general

290 population. Thus, our findings cannot be directly compared to previous scientific literature.

291 However, some studies have been conducted to investigate associations between

292 occupational, residential or domestic pesticide exposure and breast cancer risks. Studies

293 largely focused on organochlorine (OC) pesticides and related metabolites (for instance

294 Dichlorodiphenyltrichloroethane (DDT), Dichlorodiphenyldichloroethylene (DDE), β-

295 Hexachlorocyclohexane (β-HCH), Hexachlorobenzene (HCB), Pentachlorothioanisole

296 (PCTA), now banned in European Union, reporting higher breast cancer risk for users

297 (personal or occupational) [50–52].

298 Breast cancer risks were found higher with exposure to OC (use vs never use) in a study

299 published by Engel et al (2005) conducted in farmer's wives population [21]. In this study,

300 breast cancer risks also appeared elevated regarding organophosphorus pesticide family (OP)

301 as a whole. When analysis was performed on compounds taken separately, association was

302 significant only for malathion. Stratification on menopausal status was performed and showed

303 higher risks among postmenopausal women whose husbands used OC and also OP such as

304 chlorpyrifos, diazinon and malathion. In our study, NMF component 1, positively correlated

305 with malathion and chlorpyriphos (respective correlation coefficients 0.76 and 0.73), was

306   significantly associated with breast cancer risk for participants with a BMI > 25 kg/m^2.

307   Moreover, in analyses conducted in 2015 by Lerro *et al.* in the Agricultural Health Study [22],

308   spouses whose husband used OPs had higher breast cancer risk compared to spouses whose

309   husbands never used OPs (RR= 1.20, 95%CI(1.01; 1.43)). However, in that study, when

310   considering pesticides molecules separately (malathion, chlorpyriphos, terbufos), associations

311   with breast cancer risk were no longer significant, except for chlorpyriphos, and especially for

312   Estrogen Receptor Negative/Progesterone Receptor Negative (ER-/PR-) breast cancer risk.

313   These observations could be interpreted in light of some kind of synergistic effects evidenced

314   in toxicological studies when exposed to pesticide residue mixtures [6,53,54]. In the same study

315   by Lerro et al., after stratification on menopausal status, significant association between

316   higher breast cancer risks and use of any OPs was observed among the postmenopausal

317   women. Again, no significant associations between OP pesticides taken separately and breast

318   cancer risk were found. Another recent study also found elevated risks in women exposed to

319   chlorpyrifos compared with those not exposed (OR = 3.22; 95%CI(1.38,7.53) [55]. These results

320   are consistent with our results suggesting an association between NMF component 1

321   (reflecting exposure to chlorpyriphos, imazalil, malathion, thiabendazole) and

322   postmenopausal breast cancer risk.

323   It is important to note that after exclusion of 'early cases' (<1 year after baseline), an

324   important drop was observed in the HR$_{Quintile\ 5\ vs\ Quntile\ 1}$ for Component 1 from 1.73 to 1.37. It

325   is possible that excluded cases exhibited very specific nutritional and health characteristics

326   linked to their imminent diagnosis and probable health deterioration linked to it. We should

327   also note that Quintile 5 lost more cases than other quintiles (42 cases to 31) and this could

328   somehow influence the analysis.

329   In our study, possible hypotheses to explain the negative associations between NMF

330   Component 3 and postmenopausal breast cancer risk rely on the fact that besides being highly

331   correlated with some pesticides used in organic farming (i.e. natural pyrethrins, spinosad), this

332   component is also negatively correlated with several synthetic pesticides (azoxystrobin,

333   chlorpropham, methamidophos). Participants with high component 3 score, seemed generally

334   less exposed to the synthetic studied pesticides but also less exposed to pesticides with high

335   suspected toxicity such as chlorpyriphos, imazalil, malathion. These results are consistent

336   with those of another study, conducted in the NutriNet-Santé cohort in 2018, that reported a

337   negative association between high organic food score and postmenopausal breast cancer risk

338   ($HR_{Quintile4}$= 0.66; 95% CI(0.45-0.96)) [56]. One formulated hypothesis for this association was

339   that organic farming regulations lead to a lower frequency or an absence of pesticide residues

340   in organic foods compared with conventional foods [25]. Thus, our present results are consistent

341   with this hypothesis. Moreover, effects had comparable magnitude.

342   Mechanisms underlying these associations could be related to carcinogenic properties of some

343   organophosphate pesticides provoking DNA damage, cell apoptosis dysregulation, epigenetic

344   changes [57], cell signaling disruption [58], nuclear receptor binding (Aromatic hydrocarbon

345   Receptor, AhR) [59] or oxidative stress induction [4,8,9]. It can be noted that IARC classified some

346   organophosphate pesticides as "probably carcinogenic to humans" (Group 2A) and "possibly

347   carcinogenic to humans" (Group 2B) [10]. Endocrine disruption potential of pesticides has also

348   been described in toxicological studies and recently in a review by Yang et al. [7,60], and could

349   be particularly involved in hormone-dependent breast carcinogenesis, as some pesticides are

350   known to mimic estrogen functions [57,61]. Indeed, azole fungicides, including imazalil, for

351   which we found high correlations with NMF Component 1, have been related to inhibition of

352   estrogen biosynthesis in some studies [62]. These pesticides are also known to affect

353   mitochondrial activity and oxidoreduction status [63].

354   When considering stratified analyses on BMI (threshold 25 kg/m^2), a positive association

355   between component 1 and post-menopausal breast cancer risk was observed in overweight

356   individuals.

357   Several studies have found positive associations between body fat and OC pesticide blood

358   levels, overweight subjects having higher blood levels of these pesticides [64,65]. However, it is

359   unlikely this would be the case for OPs, which are not accumulated in adipose tissue.It is

360   possible that there is cumulative effect between obesity and pesticide exposure on cancer

361   risks. The specific association in overweight women could also be explained by differences in

362   paraoxonase 1 (PON1) activity, as this enzyme is involved in lipid metabolism, but also

363   participates in hydrolysis of organophosphate compounds [66]. Indeed, some studies have

364   shown lower levels of PON1 activity in overweight and obese patients [67,68]. In consequence,

365   toxicity of these pesticides could be higher for this subgroup.

366   More data are needed on OP pesticides, in order to fully understand underlying mechanisms

367   of this association and potential modifying effects of BMI on breast cancer.

368   It should be noted that associations between NMF Components 2 and 4 and postmenopausal

369   breast cancer risk were not statistically significant in our study. Given the lack of evidence on

370   specific pesticide mixtures in relation to human health, it is difficult to know whether this

371   could be due to specific non-carcinogenic patterns of the studied pesticides or to the fact that

372   the population may not be exposed enough to experience deleterious health effects.

373   *Limitations and strengths*

374   Some limitations of this study should be mentioned. Firstly, the NutriNet-Santé cohort is

375   composed of volunteers, mostly highly educated, who can be more interested in their health

376   and dietary intakes than the general French population [69]. This implies cautions when

377   generalizing our results to other populations.

378   It is important to mention that dietary intakes were self-reported through a food frequency

379   questionnaire (FFQ) and this may have caused overestimation of organic food consumption.

380   Other limitations come from the database used to estimate dietary pesticide exposures, since

381 data were not available for animal products and the database did not contain measures for

382 copper or sulfur-based products, widely used in organic farming, but not known as

383 carcinogenic compounds. Measures were performed in Germany, but products from all over

384 European Union were tested.

385 Another limitation that should be mentioned was that the dietary pesticide exposure was

386 estimated and is therefore not as accurate as measuring biomarkers. It is to note that

387 biomarkers can rarely be measured on very large samples given feasibility and cost

388 constraints. Another disadvantage of using biomarkers is that it would not give precision on

389 the active substances to which individuals are exposed since biomarkers are not specific to

390 one molecule.

391 We should acknowledge that pesticide exposure have probably been overestimated as

392 potential concentration or dilution effects during washing, cooking or peeling on pesticide

393 residue levels were not accounted for [37].Finally, for this study, follow-up duration was short

394 and the number of cases limited, given a high estimated latency period for this type of disease.

395 This can be a limitation for causal inference and statistical power of the analyses. However,

396 we hypothesize that dietary habits change marginally over time, therefore dietary habits four

397 years before diagnosis were probably very similar to those 10 years before. In the same way,

398 patterns of pesticide use in France were similar during this period, as there were changes in

399 authorizations only for three selected pesticides (anthraquinone, methamidophos and

400 profenofos) [70]. Nevertheless, it will be interesting to reassess cancer risks after several years,

401 in order estimate long-term effects.

402 Some strengths of this study can also be put forward.

403 Cox regression models were adjusted for a wide range of covariates, including major

404 confounders such as diet quality indicators. Despite a limited number of cases, the sample size

405   still allowed us to perform some stratification and sensitivity analyses in order to deepen the

406   understanding of these results and reduce confounding bias.

407

<u>Conclusion</u>

We observed a negative association between low synthetic pesticide exposure profile (through NMF component 3) and post-menopausal breast cancer risk. Positive association between component 1 (highly correlated to chlorpyriphos, imazalil, malathion, thiabendazole) and postmenopausal breast cancer risk was also found specifically among overweight and obese women. If confirmed by other studies, some pesticides profiles may constitute risk factors among subgroups such as those with overweight. Observed associations should be investigated in other prospective studies, in different settings, coupled with experimental studies to complement these observational studies in order to validate estimated dietary pesticide exposure. A better understanding of the impact of dietary pesticides on human health could unlock prevention strategies for the whole population through regulation.

428

429   **Data availability**

430   Data of the study are protected under the protection of health data regulation set by the French

431   National Commission for Information Technology and Liberties (Commission Nationale de

432   l'Informatique et des Libertés, CNIL). The data are available upon reasonable request to the

433   study's operational manager, Nathalie Pecollo (n.pecollo@eren.smbh.univ-paris13.fr), for

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445   **Author contributions**

446   RV, DL, JB, SH and EK-G conducted the research.

447   PR performed statistical analyses and drafted the manuscript.

448   All authors critically helped in the interpretation of results, revised the manuscript and

449   provided relevant intellectual input. They all read and approved the final manuscript. EK-G

450   supervised the study, had primary responsibility for the final content, she is the guarantor.

451 **Conflict of Interest:**

452 None declared.

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Tables and Figures:

Table 1: Characteristics of the participants, NutriNet-Santé Study, 2014 (N=13,149)

	All participants	Non-cases	Cases	P[1]
N	13,149	12,980	169	
Age, mean (SD)	60.49 (7.39)	60.48 (7.40)	61.15 (6.43)	0.24
Monthly income per household unit, %				0.27
<€1200	9.29	9.30	8.88	
€1200-1800	21.63	21.69	16.57	
€1800-2700	28.09	28.11	26.04	
>€2700	33.61	33.54	38.46	
Unwilling to answer	7.38	7.35	10.06	
Educational level, %				0.17
Less than high-school diploma	26.73	26.81	20.71	
High school diploma	17.58	17.53	20.71	
Post Graduate	55.69	55.65	58.58	
Occupational status, %				0.26
Employee, manual worker	12.29	12.36	7.10	
Intermediate profession	12.91	12.90	13.61	
Managerial staff, intellectual	14.15	14.18	11.83	
Retired	48.92	48.82	56.21	
Self-employed, farmer	1.59	1.59	1.18	
Unemployed or never employed	10.14	10.14	10.06	
Place of residence, %				0.85
Rural community	22.98	23.00	21.30	
Urban unit with a population <20,000 inhabitants	15.77	15.76	16.57	
Urban unit with a population between 20,000 and 200,000	19.45	19.48	17.75	
Urban unit with a population >200,000 inhabitants	41.80	41.76	44.38	
Smoking habits, %				0.20
current smoker	9.00	9.04	5.92	
former smoker	42.29	42.33	39.64	
never smoker	48.71	48.64	54.44	
Body Mass Index (kg/m²), mean (SD)	24.22 (4.64)	24.22 (4.64)	24.57 (4.45)	0.16
Body Mass Index > 25kg/m², %	34.19	34.17	35.50	0.72
Physical activity, %				0.12
High	35.49	35.57	29.59	
Moderate	36.06	36.05	37.28	
Low	17.07	17.08	16.57	
Missing data	11.37	11.30	16.57	
Use of hormonal treatment for menopause, %				0.04
Yes	10.92	10.87	14.20	
No	83.30	83.29	84.02	
Missing data	5.78	5.83	1.78	
Parity, %				0.33
No children	14.47	14.43	17.75	
One child	17.56	17.59	15.38	
2 children	40.12	40.18	35.50	
More than 2 children	27.84	27.80	31.36	
Family history of cancer, %	52.25	52.19	57.40	0.18

[1]P-values for comparisons between cases and non-cases using Chi-square tests or Wilcoxon tests, as appropriate.

Table 2: Estimated pesticide exposure for cases and non-cases, lower-bound scenario, NutriNet-Santé Study, 2014 (N=13,149)

Variable	Non-cases N=12,980		Cases N=169	
	Mean	SD	Mean	SD
Acetamiprid	0.0598	0.0778	0.0548	0.0966
Anthraquinone	0.0005	0.0015	0.0005	0.0010
Azadirachtin	0.0004	0.0005	0.0003	0.0004
Azoxystrobin	0.0451	0.0453	0.0457	0.0520
Boscalid	0.1312	0.1114	0.1218	0.1096
Carbendazim	0.0564	0.0581	0.0535	0.0708
Chlorpropham	0.0607	0.0616	0.0674	0.0632
Chlorpyrifos	0.0753	0.0663	0.0758	0.0751
Cypermethrin	0.0881	0.1107	0.0815	0.1370
Cyprodinil	0.0821	0.0892	0.0782	0.0790
Difenoconazole	0.0190	0.0177	0.0175	0.0157
Dimethoate Ometoate	0.0106	0.0134	0.0090	0.0138
Fenhexamid	0.1067	0.1455	0.0897	0.1060
Glyphosate	0.0035	0.0048	0.0040	0.0057
Lambda-Cyhalothrin	0.0116	0.0112	0.0117	0.0122
Imazalil	0.8459	1.0395	0.9367	1.1376
Imidacloprid	0.0791	0.0750	0.0831	0.0750
Iprodione	0.1591	0.1833	0.1552	0.1706
Malathion	0.0003	0.0004	0.0003	0.0003
Methamidophos	0.0002	0.0003	0.0003	0.0005
Profenofos	0.0000	0.0001	0.0000	0.0001
Pyrethrins	0.0020	0.0017	0.0018	0.0015
Spinosad	0.1717	0.1870	0.1447	0.1660
Tebuconazole	0.0385	0.0471	0.0373	0.0399
Thiabendazole	0.2882	0.3247	0.3239	0.3322

Table 3: Spearman Correlations between 25 selected pesticides and NMF Components, NutriNet-Santé Study, 2014 (N=13,149)

Compounds	NMF Component 1	NMF Component 2	NMF Component 3	NMF Component 4
Acetamiprid	0.34	0.41	0.26	**0.87**
Anthraquinone	0.17	0.19	-0.06	0.18
Azadirachtin	-0.09	*-0.01	0.53	*-0.01
Azoxystrobin	0.59	**0.71**	-0.18	0.16
Boscalid	0.51	**0.90**	-0.13	0.19
Carbendazim	0.31	0.38	0.31	**0.89**
Chlorpropham	0.35	0.53	-0.30	0.12
Chlorpyrifos	**0.73**	0.44	0.11	**0.60**
Cypermethrin	0.29	0.27	0.36	**0.93**
Cyprodinil	0.50	**0.91**	-0.12	0.16
Difenoconazole	0.52	**0.68**	*0.02	0.47
Dimethoate Ometoate	0.36	0.42	0.26	**0.79**
Fenhexamid	0.46	**0.79**	-0.11	0.12
Glyphosate	0.38	0.45	-0.12	0.17
Imazalil	**1.00**	0.37	-0.11	0.14
Imidacloprid	0.51	0.24	0.20	0.56
Iprodione	0.52	**0.91**	-0.10	0.15
Lambda-Cyhalothrin	0.56	**0.84**	-0.08	0.24
Malathion	**0.76**	0.49	-0.10	0.17
Methamidophos	0.32	0.35	-0.19	0.17
Profenofos	**0.95**	0.38	-0.12	0.17
Pyrethrins	0.04	0.02	0.30	0.03
Spinosad	-0.07	-0.09	**0.99**	0.35
Tebuconazole	0.55	**0.84**	-0.10	0.19
Thiabendazole	**0.98**	0.36	-0.11	0.16

*p-value for Spearman correlation >0.05

NMF: Non-negative Matrix Factorization

Bold values denote correlation coefficients >0.60.

Table 4: Cox models for associations between dietary pesticide exposure and postmenopausal breast cancer risk, NutriNet-Santé Study, 2014 (N=13,149)

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	Total	P value for trend
NMF Component 1							
Number of participants	2629	2630	2630	2630	2630	13,149	
Incident Cases	27	36	34	30	42	169	
Person-years	11,385.52	11,507.50	11,566.09	11,477.81	11,266.78	57,203.70	
Model 1[1], HR (95% CI)	1	1.34 (0.81; 2.21)	1.24 (0.75; 2.06)	1.14 (0.68; 1.93)	1.73 (1.05; 2.84)		0.09
Model 2[2], HR (95% CI)	1	1.33 (0.80; 2.19)	1.25 (0.75; 2.07)	1.15 (0.68; 1.95)	1.78 (1.08; 2.93)		0.07
Model 3[3], HR (95% CI)	1	1.32 (0.80; 2.18)	1.24 (0.75; 2.06)	1.15 (0.68; 1.94)	1.77 (1.07; 2.91)		0.08
NMF Component 2							
Number of participants	2629	2630	2630	2630	2630	13,149	
Incident cases	38	43	28	26	34	169	
Person-years	11,504.90	11,453.11	11,459.27	11,499.86	11,286.57	57,203.70	
Model 1[1], HR (95% CI)	1	1.11 (0.72; 1.72)	0.72 (0.44; 1.17)	0.69 (0.42; 1.15)	0.96 (0.59; 1.56)		0.30
Model 2[2], HR (95% CI)	1	1.09 (0.70; 1.69)	0.71 (0.44; 1.16)	0.70 (0.42; 1.16)	1.00 (0.61; 1.63)		0.38
Model 3[3], HR (95% CI)	1	1.08 (0.70; 1.68)	0.71 (0.43; 1.16)	0.69 (0.42; 1.15)	0.99 (0.61; 1.62)		0.37
NMF Component 3							
Number of participants	2629	2630	2630	2630	2630	13,149	
Incident cases	47	42	23	31	26	169	
Person-years	11,304.65	11,374.78	11,437.14	11,558.22	11,528.91	57,203.70	
Model 1[1], HR (95% CI)	1	0.88 (0.58; 1.34)	0.47 (0.29; 0.78)	0.64 (0.40; 1.01)	0.57 (0.34; 0.93)		**0.006**
Model 2[2], HR (95% CI)	1	0.89 (0.59; 1.35)	0.48 (0.29; 0.80)	0.66 (0.41; 1.04)	0.59 (0.36; 0.97)		**0.01**
Model 3[3], HR (95% CI)	1	0.89 (0.59; 1.35)	0.48 (0.29; 0.80)	0.66 (0.41; 1.04)	0.59 (0.36; 0.98)		**0.01**
NMF Component 4							
Number of participants	2629	2630	2630	2630	2630	13,149	
Incident cases	36	41	33	36	23	169	
Person-years	11,294.95	11,425.20	11,459.16	11,442.46	11,581.94	57,203.70	
Model 1[1], HR (95% CI)	1	1.14 (0.72; 1.78)	0.93 (0.58; 1.50)	1.01 (0.64; 1.62)	0.65 (0.38; 1.12)		0.13
Model 2[2], HR (95% CI)	1	1.15 (0.73; 1.80)	0.95 (0.59; 1.53)	1.02 (0.64; 1.63)	0.66 (0.39; 1.12)		0.13
Model 3[3], HR (95% CI)	1	1.15 (0.73; 1.80)	0.95 (0.59; 1.53)	1.02 (0.64; 1.62)	0.66 (0.39; 1.12)		0.13

Abbreviations: NMF:Non-negative Matrix Factorization; HR: Hazard Ratio; 95% CI : 95% Confidence Interval; sPNNS-GS2: Simplified Programme National Nutrition Santé Guideline Score 2

[1]Adjusted for smoking practices, educational level, physical activity, alcohol intake, alcohol-free energy intake, Body Mass Index, height, family history of cancer, menopausal treatment, parity

[2]Adjusted for Model 1 + sPNNS-GS2 score

[3]Adjusted for Model 1 + sPNNS-GS2 score + residing currently in an agricultural area

Table 5: Cox models for associations between dietary pesticide exposure and postmenopausal breast cancer risk, stratified analyses on BMI, NutriNet-Santé Study, 2014 (N=13,149)[1]

	BMI <25kg/m² N=8654, 109 cases						BMI >25kg/m² N= 4495, 60 cases					
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5		Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	
N	1731	1732	1732	1732	1732		901	901	901	901	901	
	HR	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	P-value for trend	HR	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	P-value for trend
NMF Component 1	1	1.05 (0.60; 1.85)	0.65 (0.34; 1.23)	1.02 (0.57; 1.81)	0.98 (0.53; 1.81)	0.88	1	2.83 (0.83; 6.87)	2.58 (0.90; 7.38)	3.02 (1.08; 8.47)	4.13 (1.50; 11.44)	**0.006**

Abbreviations: NMF: Non-negative Matrix Factorization; HR: Hazard Ratio; 95% CI: 95% Confidence Interval; BMI: Body Mass Index; sPNNS-GS2: Simplified Programme National Nutrition Santé Guideline Score 2

[1]All models adjusted for sPNNS-GS2 score, smoking practices, educational level, physical activity, alcohol intake, alcohol-free energy intake, BMI, height, family history of cancer, menopausal treatment, parity.

Table 6: Sensitivity analyses for associations between dietary pesticide exposure and postmenopausal cancer risk, NutriNet-Santé Study, 2014 (N=13,149)

Model excluding early cases (1 year)	Quintile 1	Quintile 2		Quintile 3		Quintile 4		Quintile 5		
N=13,120; 140 cases	HR	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	P-value for trend
NMF Component 1	1	1.11	(0.65; 1.90)	1.19	(0.70; 2.03)	1.02	(0.58; 1.78)	1.37	(0.80; 2.36)	0.38
NMF Component 2	1	0.96	(0.60; 1.55)	0.65	(0.38; 1.11)	0.66	(0.38; 1.14)	0.86	(0.50; 1.47)	0.23
NMF Component 3	1	0.96	(0.61; 1.50)	0.46	(0.26; 0.81)	0.62	(0.37; 1.04)	0.62	(0.36; 1.07)	**0.016**
NMF Component 4	1	1.22	(0.75; 2.01)	1.15	(0.69; 1.91)	1.00	(0.59; 1.69)	0.59	(0.32; 1.10)	0.10
Model with additional adjustment for ultra-processed foods[2]	Quintile 1 N=2629	Quintile 2 N=2630		Quintile 3 N=2630		Quintile 4 N=2630		Quintile 5 N=2630		
N=13,149; 169 cases	HR	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	P-value for trend
NMF Component 1	1	1.33	(0.80; 2.19)	1.24	(0.75; 2.07)	1.15	(0.68; 1.94)	1.77	(1.08; 2.92)	0.08
NMF Component 2	1	1.09	(0.70; 1.69)	0.71	(0.43; 1.16)	0.70	(0.42; 1.16)	0.99	(0.61; 1.62)	0.37
NMF Component 3	1	0.88	(0.58; 1.34)	0.47	(0.29; 0.79)	0.64	(0.40; 1.03)	0.58	(0.35; 0.96)	**0.009**
NMF Component 4	1	1.15	(0.73; 1.80)	0.94	(0.59; 1.52)	1.01	(0.64; 1.62)	0.65	(0.38; 1.11)	0.12
Model with additional adjustment for provegetarian Score[3]	Quintile 1 N=2629	Quintile 2 N=2630		Quintile 3 N=2630		Quintile 4 N=2630		Quintile 5 N=2630		
N=13,149; 169 cases	HR	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	P-value for trend
NMF Component 1	1	1.33	(0.80; 2.19)	1.23	(0.74; 2.04)	1.13	(0.67; 1.91)	1.72	(1.04; 2.82)	**0.009**
NMF Component 2	1	1.09	(0.70; 1.70)	0.71	(0.43; 1.16)	0.69	(0.41; 1.14)	0.96	(0.59; 1.56)	0.30
NMF Component 3	1	0.88	(0.58; 1.34)	0.47	(0.29; 0.78)	0.64	(0.40; 1.02)	0.57	(0.34; 0.95)	**0.008**
NMF Component 4	1	1.14	(0.73; 1.79)	0.94	(0.58; 1.51)	1.01	(0.64; 1.62)	0.65	(0.38; 1.12)	0.12

Abbreviations: NMF: Non-negative Matrix Factorization; HR: Hazard Ratio; 95 % CI: 95% Confidence Interval; BMI: Body Mass Index; sPNNS-GS2: Simplified Programme National Nutrition Santé Guideline Score 2

[1]Adjusted for sPNNS-GS2 score, smoking practices, educational level, physical activity, alcohol intake, alcohol-free energy intake, BMI, height, family history of cancer, menopausal treatment, parity

[2]Adjusted for sPNNS-GS2 score, smoking practices, educational level, physical activity, alcohol intake, alcohol-free energy intake, BMI, height, family history of cancer, menopausal treatment, parity and percentage of ultra-processed foods in the diet

[3]Adjusted for provegetarian score, smoking practices, educational level, physical activity, alcohol intake, alcohol-free energy intake, BMI, height, family history of cancer, menopausal treatment, parity