The associations of the Palaeolithic diet alone and in combination with lifestyle factors with type 2 diabetes and hypertension risks in women in the E3N prospective cohort

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Title: The associations of the Palaeolithic diet alone and in combination with lifestyle factors with type 2 diabetes and hypertension risks in women in the E3N prospective cohort.

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Declarations:

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Authors' Contributions: SS, NL MCBR and GS conceived and designed the study. SS performed the statistical analysis and drafted the original manuscript. All authors contributed to the interpretation of data discussed in the manuscript, revised the manuscript and approved its final version to be published. NL is the guarantor of this work and, as such, has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Short Running Head: Palaeolithic diet, diabetes and hypertension

Abbreviations:

AHA- American Heart Association

BMI- Body mass index

CI- Confidence interval

DASH- Dietary Approach to Stop Hypertension

E3N- Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale

HDL- High-density lipoproteins

HR- Hazard ratio

LDL- Low-density lipoproteins

MGEN- Mutuelle Générale de l'Education Nationale

NCDs- Non-communicable diseases

PD- Palaeolithic diet

SD- Standard deviation

T2D- Type 2 diabetes

WHO- World Health Organization

US- United States

Registry: The protocol is registered at clinicaltrials.gov as NCT03285230

Characters: 49, 983

1   Abstract

2   Purpose: Patterns of change from the traditional Palaeolithic lifestyle to the modern lifestyle

3   may partly explain the epidemic proportions of non-communicable diseases (NCDs). We

4   investigated to what extent adherence to the Palaeolithic diet (PD) and the Palaeolithic-like

5   lifestyle was associated with type 2 diabetes (T2D) and hypertension risks.

6   Methods: A study of 70,991 women from the E3N (*Etude Epidémiologique auprès de*

7   *femmes de la Mutuelle Générale de l'Education Nationale*) cohort, followed up for nearly 20

8   years. There were 3,292 incident T2D and 12,504 incident hypertension cases that were

9   validated. Dietary data were collected at baseline in 1993 via a food frequency questionnaire.

10   The PD score and the Palaeolithic-like lifestyle score (PD, physical activity, smoking status

11   and body mass index [BMI]) were derived and considered in quintiles. Multivariable Cox

12   regression models were employed to estimate hazard ratios (HR) and 95% confidence

13   intervals (CI) for incident T2D and hypertension.

14   Results: In the fully adjusted models, a 1-SD increase of the PD score was associated with

15   4% and 3% lower risks of T2D and hypertension, respectively. Those in the highest versus

16   the lowest quintile of the score had HR (95% CI) of 0.88 (0.79, 0.98) and 0.91 (0.86, 0.96)

17   for T2D and hypertension, respectively (*P*-trend <0.0001). Associations were stronger for the

18   Palaeolithic-like lifestyle score; in the fully adjusted model a 1-SD increase of the score was

19   associated with 19% and 6% lower risks of T2D and hypertension, respectively. Risks

20   lowered successively with each increase in quintile; those in the highest versus the lowest

21   quintile had HR (95% CI) of 0.58 (0.52, 0.65) and 0.85 (0.80, 0.90) for T2D and

22   hypertension, respectively (*P*-trend < 0.0001).

23   Conclusions: Our data suggest that adhering to a PD based on fruits, vegetables, lean meats,

24   fish, and nuts, and incorporating a Palaeolithic-like lifestyle could be promising options to

25   prevent T2D and hypertension.

26   Keywords: Dietary pattern, The Palaeolithic diet, Palaeolithic-like lifestyle, Type 2 diabetes,

27   Hypertension, Prospective study

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42 Introduction

43 Over the last two to three decades, non-communicable diseases (NCDs) such as type 2

44 diabetes (T2D) and hypertension have become the most prevalent and costly health

45 conditions in the developed world [1, 2]. According to the International Diabetes Federation,

46 the prevalence of T2D is on the rise; from 415 million adults in 2015 worldwide, it is

47 expected to increase to 700 million adults by 2045 [3]. Furthermore, the World Health

48 Organization (WHO) has estimated the prevalence of hypertension to rise 29% by 2025 from

49 a current prevalence of 972 million people which constitutes roughly 30% of the World's

50 population [3–5]. These NCDs are strongly linked with cardiovascular diseases, the leading

51 causes of morbidity and mortality globally [4]. Whilst the worldwide aging phenomenon is

52 the main driver of the development of NCDs [6, 7], literature indicates the concurrent

53 occurrence of changes in diet and lifestyle [6]. Hence, it is speculated that a shift back to the

54 traditional lifestyle could avert this epidemic [8].

55 Studies have attributed lifestyle-related chronic diseases to modern behaviours which include

56 increased sedentary time, smoking, and behaviours leading to excess energy intake and

57 adiposity [9]. Furthermore, the Western diet, rich in processed meat, high-fat dairy, and

58 refined products, is associated with increased production of oxygen species and oxidative

59 stress which leads to T2D and hypertension [10, 11]. In contrast, traditional lifestyle patterns

60 that resemble the culture of Palaeolithic people characterised by high levels of physical

61 activity, energy balance which results in lean body mass, non-smoking [12, 13], and diets

62 metabolically more attuned to traditional patterns of human evolution composed of lean meat,

63 fish, fruit, vegetables, and nuts, with exclusion of processed foods, dairy products, and

64 refined cereal products, the 'Palaeolithic-like diets' [14–16], could potentially prevent or

65 reverse these disorders.

66   Palaeolithic nutrition has emerged as one of the most popular dietary patterns worldwide with

67   few prospective studies to date. Whalen et al. constructed a Palaeolithic diet (PD) score as

68   discordance between diet during the Palaeolithic period and that during the present era [17,

69   18]. Because the cumulative association between adverse diet and lifestyle factors, this score

70   has been studied alone or in combination with lifestyle factors in relation to incident

71   colorectal adenomas [17] and mortality [18] with findings of inverse associations. To be best

72   of our knowledge, no study has examined the potential associations of long-term adherence

73   to the PD with T2D and hypertension risks. Although the concept of the Palaeolithic nutrition

74   is not novel, more studies with larger sample size and longer follow-up period were required

75   to identify the PD as a healthier dietary pattern and an alternative that could be incorporated

76   in future nutritional guidelines for prevention of NCDs.

77   Therefore, we aimed to investigate the extent of the influence of the PD on T2D and

78   hypertension risks in the French E3N prospective cohort study of women. In addition, we

79   aimed to clarify whether a higher adherence to an overall Palaeolithic-like lifestyle would

80   contribute to benefits over and above those of the PD alone.

81   Materials and Methods

82   E3N cohort and Study Population for Analysis

83   The E3N (*Etude Epidémiologique auprès de femme de la Mutuelle Générale de l'Education*

84   *Nationale*) is a large-scale French prospective cohort study initiated in 1990. It comprised of

85   98,995 women, aged 40-65 years at baseline and affiliated with the French national health

86   insurance plan (*Mutuelle Générale de l'Education Nationale*, MGEN) for personnel of the

87   French education system and their families. Participants were enrolled after informed written

88   consent. Data were collected at inclusion and every two to three years by self-administered

89   questionnaires (average response rate per questionnaire of approximately 83%). Detailed

90   information about health conditions, lifestyle, diet, treatments, etc. were recorded.

91   Furthermore, all outpatient reimbursements for health expenditure since January 1, 2004 for

92   each participant were obtained through the health insurance plan. These data included brand

93   names, doses, and dates of drug reimbursements. The study received ethical approval from

94   the French National Commission for Data Protection and Privacy (Commission Nationale

95   Informatique et Libertés). The protocol is registered at clinicaltrials.gov as NCT03285230

96   Eligible participants (n = 74,522) completed and returned the baseline dietary questionnaire

97   sent in 1993. The 1993 data served as the baseline and the cohort follow-up ended in 2014

98   (latest date of T2D and hypertension case validation in the E3N cohort). For the current

99   study, we used data from two sub-samples (Supplementary Figure 1). First, we excluded the

100   following participants: women with extreme energy intakes (i.e. below the 1st and above the

101   99th percentiles of the energy intake over energy requirement distribution in the population)

102   (n = 1,774) and women who did not complete any follow-up questionnaire after the dietary

103   questionnaire (n = 1,216). Then, for each outcome of interest, all prevalent cases (n = 824 and

104   n = 28,164 for T2D and hypertension, respectively), i.e. cases reported to have occurred

105   before the baseline date for the present study, i.e. the date when the 1993 questionnaire was

106   answered, were excluded. This resulted in a final study size of 70,991 and 46,075 participants

107   for T2D and hypertension analyses, respectively.

108   The Palaeolithic diet score

109   Dietary data were collected through a validated [19, 20] self-administered food frequency

110   questionnaire with quantitative questions about consumption frequencies for eight eating

111   moments from breakfast to after dinner snacks, portion sizes facilitated by photographs, and

112   qualitative questions concerning specific food and drink items structured according to French

113   meal patterns. Participants recalled intake of food and beverages over the preceding 12

114   months. All food intakes were converted into daily intakes of energy and nutrients by means

115   of a study-specific Food Composition Database derived from the French Information Center

116   on Food Quality [21] .

117   A score reflecting adherence to the PD was employed as described previously by Whalen et

118   al. [17]. Briefly, all food items were categorised into 14 food groups. These food groups were

119   classified as more characteristic of the PD (vegetables, fruits, fruit and vegetable diversity

120   score, lean meat, fish, nuts, and calcium), and less characteristic of the PD (red and processed

121   meat, dairy foods, sugar sweetened beverages, baked goods, grains and starches, sodium, and

122   alcohol) (Table 1). Dietary diversity is a qualitative measurement of the spectrum of foods

123   consumed and is a proxy indicator of nutrient adequacy [22]. In our study, the fruit and

124   vegetable diversity score represented the number of components of the fruit and vegetable

125   group reported to be consumed by each participant. For dietary scoring, intake of each food

126   component was categorised as quintiles in accordance with the study population intake

127   distributions. Values of 1 to 5 were assigned to each quintile with higher values given to

128   higher intake of food components considered characteristic of the PD and lower values given

129   to higher consumption of foods considered less characteristic of the PD. The possible PD

130   score ranged from 14 to 70 and higher scores indicated higher levels of adherence to the PD.

131   The Palaeolithic-like lifestyle score (combined score)

132   Lifestyle factors including physical activity, smoking status, and BMI were obtained at

133   baseline and follow-up questionnaires. Physical activity was assessed through six questions

134   based on usual distance walked daily (<500, 500-2,000, and ≥2,000 m), the average number

135   of flights of stairs climbed daily (0, 1-4, and ≥5), the average amount of time spent weekly

136   doing light household activity (0, 1-4, 5-13, and ≥14 hours) and heavy household activity (0,

137   1-4, and ≥5 hours), and the average amount of time spent weekly doing moderate recreational

138   activity (0, 1-4, 5-13, and ≥14 hours) and vigorous recreational activity (0, 1-4,and ≥5 hours).

139   A total physical activity score was calculated by multiplying the metabolic equivalent of task

140   (MET-hours/week) of each activity by their frequency and duration. For smoking status,

141   participants were classified smoker, ex-smoker and non-smoker. Self-reported height and

142   weight at baseline were used to calculate BMI, defined as weight (kilograms) divided by

143   squared height (meters^2). In the E3N cohort, self-reported anthropometry is considered

144   reliable from a validation study [23]. In line with an existing 'evolutionary-concordance

145   lifestyle score' developed by Cheng et al., which reflected the overall 'Palaeolithic diet and

146   lifestyle score', we combined BMI, physical activity, smoking status, and the PD score to

147   construct the 'Palaeolithic-like lifestyle score' [24]. Briefly, participants were categorised

148   according to the quintile and tertile distributions of the PD score and lifestyle factors (except

149   smoking status), respectively. For the lower quintile group of the PD corresponding to the

150   lower adherence, value of 1 was assigned and one additional point was given for each

151   increasing quintile. For physical activity, participants in the highest tertile were given values

152   of 5 whereas values of 3 and 1 were given for each decreasing tertile, respectively; in

153   contrast, the scoring was reversed for BMI. For smoking status, value of 5 was assigned to

154   non-smokers and values of 3 and 1 for ex-smoker and smokers, respectively. Finally, all diet

155   and lifestyle values for each participant were summed to reflect adherence to the Palaeolithic-

156   like lifestyle score. In our cohort, the final score ranged from 17 to 85 and higher scores

157   indicated better adherence.

158   Outcome ascertainment

159   Incident T2D was ascertained through self-reports, validation questionnaires and drug

160   reimbursement files from 1993 until last validation of cases in 2014. The detailed procedure

161   has been described elsewhere [25]. Before 2004, all potential cases of T2D were identified

162   through follow-up questionnaires that included questions on the diagnosis of T2D, diabetes-

163   specific diets, diabetes drugs and hospitalisations for T2D. All potential cases were then

164   contacted and asked to respond to a diabetes-specific questionnaire that included questions on

165   the circumstances of diagnosis (year of diagnosis, symptoms, biological

166   examinations, and fasting or random glucose concentration at diagnosis), T2D therapy

167   (prescription of diet or physical activity, list of all glucose-lowering drugs already used), and

168   the most recent concentrations of fasting glucose and HBA1c. The validation was based on

169   WHO criteria as follows: fasting glucose of 7.0 mmol/L (126 mg/dL) or random glucose of

170   11.1 mmol/L (200 mg/dL)] and/or reported diabetic drugs, and/or last values of fasting

171   glucose or glycated haemoglobin concentrations reported to be 7.0 mmol/L (126 mg/dL) or

172   7%, respectively. After 2004, T2D cases were identified through a drug reimbursement

173   database. All women with at least two reimbursements for any glucose-lowering medication

174   within a 1-year period were considered to be validated T2D cases, with the date of diagnosis

175   defined as the date of their first reimbursement.

176   For hypertension, participants were asked to report whether they had hypertension at baseline

177   (1993) and in each follow-up questionnaire (1995, 1997, 2000, 2002, 2005, 2008, 2011, and

178   2014), the date of diagnosis, and the use of antihypertensive treatments. The month and year

179   of diagnosis were provided for most cases (69%). For individuals who were missing the

180   month of diagnosis (14% of cases), it was imputed to June of the year of diagnosis. The

181   median time between the date of diagnosis and the date of response to the first questionnaire

182   after diagnosis was 12 months. Thus, for cases with no year of diagnosis (n = 17%), we

183   assigned it to be 12 months before they reported hypertension in a questionnaire. From 2004,

184   a drug reimbursement database became available for 97.6 % of participants. We used the

185   self-reported date of diagnosis or the first date of drug reimbursement for antihypertensive

186   medications (Anatomical Therapeutic Chemical Classification System codes C02, C03, C07,

187 C08, and C09) whatever happened first, as the date of diagnosis for cases identified after

188 2004. In addition, using the information of the MGEN health insurance plan drug claim

189 database, we assessed the validity of self-reported hypertension within the E3N cohort. We

190 compared hypertension self-report to antihypertensive drug reimbursement (any of the above

191 specified codes). A positive predictive value of 82% was observed among women alive in

192 January 2004 and followed up to their response to the last considered questionnaire in 2008

193 [26].

194 Covariates

195 Potential confounders of the association between the PD and T2D and hypertension risks

196 were accounted for after literature review of their associations with both exposure and

197 outcomes. They consisted of baseline self-reported sociodemographic and anthropometric

198 characteristics, total energy intake, physical activity, smoking status, BMI, personal history

199 of hypercholesterolemia, and family history of diabetes and hypertension.

200 Statistical Analyses

201 Description of participants' baseline characteristics according to T2D and hypertension status

202 across quintiles of the PD and the combined PD and lifestyle scores were described using

203 mean (standard deviation [SD]) for continuous variables, and frequencies and proportions for

204 categorical variables. Cox proportional hazards regression models with age as the time-scale

205 were used to estimate hazards ratios (HR) and 95% confidence intervals (CI). Participants

206 were followed from age at baseline until age at diagnosis of T2D or hypertension, or age at

207 death, or age at last follow-up, or age at the end of the follow-up period (2014 for T2D and

208 hypertension), whichever occurred first. The proportional hazards assumption was tested

209 graphically using Schoenfeld Residuals. No major violations were observed.

210   All scores were modelled in three ways. First, as continuous variable where we reported

211   estimates for a 1-SD increase assuming a linear association between the scores and incidence

212   of health events. Second, to check this assumption we used multivariate restricted cubic

213   splines with fives knots placed at the 5th, 27.5th, 50th, 72.5th and 95th percentiles of each

214   score to provide a graphical representation [27]. Splines allowed us to test whether there was

215   a significant departure from a linear association. Finally, all scores were categorised into

216   quintiles, and the first quintile group was considered as the reference category to assess

217   whether subsequently higher categories were associated with lower risks of T2D and

218   hypertension.

219   Main models of associations between the PD and risks of T2D and hypertension were

220   adjusted for the following potential confounders: age (as the time-scale), educational level (<

221   Baccalaureate, Baccalaureate to Baccalaureate +2, > Baccalaureate +2), smoking status

222   (non-smoker, past, current), BMI (continuous), physical activity (continuous), family history

223   of T2D (yes, no [T2D model]), family history of hypertension (yes, no [hypertension model]

224   ), personal history of hypercholesterolemia (yes, no) and energy intake (continuous). The

225   main models for the associations between the combined score and T2D and hypertension

226   risks were not adjusted for BMI, smoking status and physical activity since these variables

227   were used to build the combined score. Missing values were less than 5% for all variables

228   and were either assigned to the median for continuous variables or the mode for categorical

229   variables.

230   Sensitivity analyses

231   Two sensitivity analyses were performed. First, our main analyses were repeated after

232   excluding women with T2D and hypertension diagnosed in the first 5 years of follow-up to

233   examine potential reverse causation. Finally, we further adjusted the main models for

234   adherence to the Western dietary pattern [28].

235   All analyses were conducted using SAS statistical software, version 9.4 (SAS Institute, Inc.,

236   Cary, North Carolina).

237   Results

238   Baseline characteristics

239   Over a mean of 18.81 (SD 4.3) years of follow-up, 3,292 (4.64%) and 12,504 (27.14%)

240   validated incident T2D and hypertension cases were identified, respectively. Table 2 and

241   Supplementary Tables 1 to 3 present the baseline characteristics of the study, overall and

242   according to quintiles of the PD and the combined score. Participants had a mean (SD) age of

243   53 (6.7) years at baseline. In general, higher adherence to the PD score and the combined PD

244   and lifestyle score was associated with older age, higher physical activity, and increased

245   frequencies of non-smoking and hypercholesterolemia at baseline.

246   The Palaeolithic diet score and type 2 diabetes and hypertension risks

247   Table 3 provides HRs of the associations between the PD, and T2D and hypertension risks.

248   After controlling for all covariates in the fully adjusted Model 3, the HR (95% CI) per SD

249   increase for T2D was 0.96 (0.93, 1.00). Similarly, there was a modest reduction in the HR

250   (95% CI) per SD increase for hypertension in Models 1 and 2, 0.98 (0.96, 1.00), which

251   became slightly stronger in Model 3, 0.97 (0.95, 0.99). Spline variables analysis did not show

252   departures from linear associations ($P = 0.920$ and $P = 0.928$ for T2D and hypertension,

253   respectively (Supplementary Figure 2). Higher quintiles of the PD score were associated with

254   lower risks of incident T2D (Model 3, P-trend <0.0001) and hypertension (Model 1: P-trend

255   0.029, Model 2: P-trend 0.019, and Model 3: P-trend 0.0001). For T2D, after full adjustment,

256   those in the highest quintile had a 12% lower risk [HR (95% CI): 0.88 (0.79; 0.98)]. For

257   hypertension, after full adjustment, those in the highest quintile had a 9% lower risk [HR

258   (95% CI): 0.91 (0.86, 0.96)].

259   The Palaeolithic-like lifestyle score (combined score) and type 2 diabetes and hypertension

260   risks

261   Table 4 provides HRs of the associations between the PD score combined with lifestyle

262   factors, and T2D and hypertension risks. The combined score was much more strongly

263   inversely associated with T2D and hypertension risks in both Models 1 and 2. After

264   controlling for all covariates in Model 2, there was a 19% lower risk of T2D and 6% lower

265   risk of hypertension [HR (95% CI) per SD increase: 0.81 (0.79, 0.84) and 0.94 (0.92, 0.95),

266   for T2D and hypertension, respectively]. There was no departure from a linear association [P

267   = 0.983 and P = 0.518 for T2D and hypertension, respectively (Supplementary Figure 3)].

268   When the combined score was categorised into quintiles, we observed a gradual inverse

269   association from quintile 1 to 5 across models for both outcomes (P-trend <0.0001). For

270   T2D, in Model 2, those in the highest versus lowest quintile had a 42% lower risk [HR (95%

271   CI): 0.58 (0.52, 0.65)]. The association was not as strong for hypertension risk with only a

272   15% lower risk for those in the highest versus lowest quintile [HR (95% CI): 0.85 (0.80,

273   0.90)].

274   Sensitivity analyses

275   Results were not substantially modified when participants with T2D and hypertension diagnosed in

276   the first 5 years of follow-up (n = 383 and n = 2910 for T2D and hypertension, respectively) were

277   excluded, and further adjustment made for the Western dietary pattern (data not tabulated).

278   Discussion

279 Considering the growing popularity of the PD, we delineated its role in the prevention of

280 NCDs. To the best of our knowledge, this study is the first to assess the association of

281 adherence to the PD alone or combined with other lifestyle factors, with T2D and

282 hypertension risks. Findings from this large-scale prospective cohort followed for

283 approximately 20 years indicated inverse linear associations between adherence to the PD

284 and incidences of both T2D and hypertension. Furthermore, adherence to the Palaeolithic-like

285 lifestyle was strongly inversely associated with risks of both T2D and hypertension,

286 independently of most known or potential risk factors or confounders. In comparison to the

287 previously reported short term beneficial effects of adherence to the PD, such as favourable

288 body weight and metabolic balance [15, 29–31], these findings provide evidence of potential

289 long-term benefits on T2D and hypertension risks.

290 Prior studies on the PD have been conducted on high risk populations such as T2D patients

291 and despite the heterogeneity in the PD definition, have reported benefits. When compared

292 with the diabetic diet, the PD was associated with a larger reduction in HBA1c, triglycerides,

293 blood pressure, and weight; however, no difference in reduction of fasting plasma glucose

294 (FPG) were seen [29]. Furthermore, when compared with the usual diet, the PD was

295 associated with lower plasma insulin, low-density lipoproteins (LDL), and triglycerides [15],

296 but not associated with high-density lipoproteins (HDL) and FPG [32]. Last, in contrast to

297 Nordic Nutritional Recommendations, there was no reduction in FPG and insulin levels [33].

298 The PD most closely resembles a low carbohydrate diet in composition [34]. Low

299 carbohydrate diets with potentially higher protein and fat intake as compared to

300 carbohydrates have been reported to be inversely associated with T2D risk [35] as well as

301 associated with beneficial glucose control in T2D patients [36]. In addition, there is systolic

302 and diastolic blood pressure reduction, weight loss, and improvement in lipid profile [37, 38].

303 In our study, the PD was positively correlated to Mediterranean diet ($r = 0.33$; $P < 0.001$) and

304   negatively correlated to Western diet (r = -0.42; $P < 0.001$) and our findings are consistent

305   with previous studies that examined these dietary patterns in relation with risks of T2D [39,

306   40] and hypertension [41–43].

307   In recent years, the role of combined lifestyle factors on the risk of NCDs has been

308   emphasised and our findings concur with studies from France and the US. A study in the E3N

309   cohort in France reported that one-third of T2D cases could be attributed to high adherence to

310   a Western diet, and that a large proportion of cases could be avoided by adopting a healthy

311   lifestyle alone [44]. Another study from the US assessed the impact of a modifiable lifestyle

312   score consisting of five culturally appropriate lifestyle factors, namely physical activity,

313   television watching, American Heart Association (AHA) healthy diet score, smoking, and

314   sleep apnea syndrome; there was a 17% lower risk of T2D over eight years of follow-up [45].

315   Moreover, a systematic review reported an approximately 75% reduction in risk of T2D by

316   adopting the healthiest lifestyle which comprised of a combination of multiple healthy

317   lifestyle factors [46]. A similar concept has been applied in hypertension research. A study in

318   the French population evaluated the impact of a combination of healthy lifestyle factors

319   namely BMI, physical activity, alcohol consumption, and the Dietary Approach to Stop

320   Hypertension (DASH) style diet score on the risk of hypertension, and reported a 65%

321   reduced risk of hypertension [47]. Although there are empirical differences in the

322   construction of various combinations of lifestyle scores and dietary patterns, we detected a

323   trend towards inverse relationships between T2D and hypertension, and healthy lifestyle

324   indices. Interestingly, when lifestyle factors were analysed individually, we observed weak

325   associations with T2D and hypertension. The strongest associations were observed when

326   combining these lifestyle factors, suggesting synergistic associations on T2D and

327   hypertension.

328   Prior studies have proposed potential mechanisms for the beneficial associations of the PD. It

329   has been hypothesized that insulin resistance emerged as an adaptation to low carbohydrate

330   foods consumed during the Palaeolithic period [32]. Moreover, population differences,

331   individual polymorphisms, and genetic variants promote insulin resistance which is

332   aggravated by consumption of high glycaemic index foods [48]. Diet has undergone

333   significant changes since the Palaeolithic period unlike the human genome, which has varied

334   slightly [49, 50]. In this regard, the PD which is devoid of high glycaemic index foods,

335   processed foods, and fats which increase extracellular acidity, could be potentially protective

336   against inflammation. High intakes of micronutrients such as magnesium, vitamins C and E,

337   carotenoids, and dietary fibre, and low intakes of saturated fats are protective as well [51].

338   Indeed animal experiments have found beneficial effects of the PD on weight, fat deposits,

339   inflammatory markers, blood pressure, and insulin sensitivity [52]. Furthermore, the PD is

340   marked by low sugar consumption, fruits and honey being the main sources of simple sugars.

341   This elicits an improvement in the metabolic profile via increased HDL as a consequence of

342   loss of fat, low glycaemic load, and higher levels of long chain fatty acids [53]. Lastly, the

343   PD enhances satiety with subsequent low energy intake via incretin and anorectic gut

344   hormones as well as by consumption of low energy-density foods such as fruits, vegetables,

345   lean meat and fish, and water [54, 55].

346   As regards the association of the combined score of diet and lifestyle factors, each modifiable

347   risk factor has been reported to exert an independent influence on the risk of disease [56–59].

348   Adiposity has demonstrated a strong association with incident diabetes, and physical activity,

349   healthy diet, non-smoking, and moderate use of alcohol have been reported to have

350   associations independent of adiposity [60]. Besides individual risk factors, each additional

351   factor and a combination of different lifestyle factors which included physical activity,

352 limited alcohol intake, and a healthy diet have been associated with a significant reduction in

353 risk of hypertension as well [47].

354 Our study has the following strengths: first, we derived the PD score and the Palaeolithic-like

355 lifestyle score to investigate their associations in relation to two major NCDs, T2D and

356 hypertension. Such scoring reflects the overall dietary and lifestyle patterns and the risks of

357 diseases and is more intuitive in understanding associations. Second, the prospective nature

358 of our study allowed assessment of NCDs incidence in a large sample with a long follow-up

359 period. Last, validated cases of T2D and hypertension minimized bias related to recall and

360 inclusion of false positive cases.

361 Limitations of our study merit further discussion. First, diet was assessed on one set of

362 responses collected in 1993 therefore any changes in diet over the follow-up period were not

363 accounted for. Second, the use of self-reported questionnaires may have led to non-

364 differential misclassification of dietary exposure as reported in the literature [61]. Third,

365 although the main potential confounding factors were accounted for, there could be residual

366 confounding due to unadjusted lifestyle factors [62]. Last, the findings would need to be

367 generalized with caution as the cohort consisted of women with presumably good levels of

368 education and health awareness hence confirmation in other populations is required.

369 However, assuming causality of findings, we would expect stronger protective associations of

370 the PD and the Palaeolithic-like lifestyle based on the dietary and lifestyle habits of the

371 general population.

372 Conclusions

373 In conclusion, our findings suggest that the PD and the Palaeolithic-like lifestyle patterns are

374 associated with lower risks of T2D and hypertension. The PD based on fruit, vegetables, lean

375 meat, fish, and nuts, with low intakes of dairy, grains, and salt, may have a favourable role in

376   the prevention of chronic diseases of the modern era. These findings also support existing

377   guidelines of behavioural lifestyle interventions for the prevention or delay of non-

378   communicable chronic diseases. Further studies are required to validate findings in other

379   population groups and to delineate the precise mechanisms of the favourable associations

380   with the PD.

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Tables

Table 1. Constituents and construction of the Palaeolithic diet score.[1]

Food category	Palaeolithic diet score[2]
More characteristic of diet	
Vegetables	Highest intake "best"
Fruits	Highest intake "best"
Fruit and vegetable diversity score	Highest intake "best"
Lean meat[3]	Highest intake "best"
Fish	Highest intake "best"
Nuts	Highest intake "best"
Calcium	Highest intake "best"
Less characteristic of diet	
Red and processed meat[4]	Lowest intake "best"
Dairy foods[5]	Lowest intake "best"
Sugar sweetened beverages	Lowest intake "best"
Baked goods[6]	Lowest intake "best"
Grains and starches[7]	Lowest intake "best"
Sodium	Lowest intake "best"
Alcohol	Lowest intake "best"

[1]All food components were in grams/day except calcium and sodium which were in milligrams/day. Highest intake best: points were assigned to each quintile; highest and lowest quintiles scored 5 and 1 points, respectively. For lowest intake best, the scoring was reversed; highest and lowest quintiles scored 1 and 5 points, respectively.

[2]The Palaeolithic diet score had 14 components and possible scores ranged from 14-70.

[3]Lean meat: poultry, rabbit, horse and veal.

[4]Red and processed meat: sheep, pork and beef.

[5]Dairy: milk, yoghurt and cheese, cream and ice cream.

[6]Baked goods: cakes, pastries, biscuits and crispbreads.

[7]Grains and starches: different sources of grains and starches including potatoes

Table 2. Baseline characteristics of type 2 diabetes sub-sample by quintile of the Palaeolithic diet score (n=70,991).

		Quintile of the Palaeolithic diet score				
	Overall (n=70,991)	Q1 (n= 13,075)	Q2 (n= 17,689)	Q3 (n= 10,508)	Q4 (n= 14,034)	Q5 (n= 15,685)
The Palaeolithic diet score mean (SD)	43.33 (5.33)	35.51 (2.48)	40.63 (1.10)	43.50 (0.50)	45.94 (0.81)	50.44 (2.36)
Type 2 diabetes (%)	3,292 (4.64)	621 (4.75)	789 (4.46)	465 (4.43)	675 (4.81)	742 (4.73)
Hypertension (%)	36,590 (51.54)	6,701 (51.25)	9,086 (51.37)	5,396 (51.35)	7,292 (51.96)	8,115 (51.74)
Age at baseline (years)	52.88 (6.67)	51.61 (6.55)	52.39 (6.58)	52.94 (6.64)	53.43 (6.68)	53.97 (6.65)
Educational level (%)						
< Baccalaureate	7,879 (11.09)	1,523 (11.65)	1,954 (11.05)	1,136 (10.81)	1,532 (10.92)	1,734 (11.06)
Baccalaureate and Baccalaureate +2	37,707 (53.12)	6,525 (49.90)	9,252 (52.30)	5,536 (52.68)	7,706 (54.91)	8,688 (55.39)
> Baccalaureate and Baccalaureate +2	25,405 (35.79)	5,027 (38.45)	6,483 (36.65)	3,836 (36.51)	4,796 (34.17)	5,263 (33.55)
Smoking status (%)						
Current	9,581 (13.50)	2,212 (16.92)	2,580 (14.59)	1,312 (12.49)	1,695 (12.08)	1,782 (11.36)
Past	23,257 (32.76)	4,229 (32.34)	5,765 (32.59)	3,493 (33.24)	4,587 (32.68)	5,183 (33.04)
Non-smoker	38,153 (53.74)	6,634 (50.74)	9,344 (52.82)	5,703 (54.27)	7,752 (55.24)	8,720 (55.60)
Physical activity (MET-h/week)	49.37 (50.48)	45.90 (43.86)	48.42 (51.73)	49.42 (47.44)	50.65 (51.87)	52.14 (54.57)
BMI (kg/m^2)	22.89 (3.18)	22.82 (3.27)	22.79 (3.18)	22.82 (3.12)	22.91 (3.14)	23.08 (3.16)
BMI (%)						
< 20 kg/m^2	10,256 (14.45)	2,071 (15.84)	2,687 (15.19)	1,534 (14.60)	1,970 (14.04)	1,994 (12.71)
≥ 20- 24 kg/m^2	46,872 (66.03)	8,516 (65.13)	11,635 (65.78)	7,054 (67.13)	9,288 (66.18)	10,379 (66.17)
≥ 25 kg/m^2	13,863 (19.52)	2,488 (19.03)	3,367 (19.03)	1,920 (18.27)	2,776 (19.78)	3,312 (21.12)
Family history of diabetes	7,882 (11.10)	1,420 (10.86)	1,893 (10.70)	1,230 (11.71)	1,523 (10.85)	1,816 (11.58)
Hypercholesterolemia (%)	5,030 (7.09)	734 (5.61)	1,110 (6.28)	751 (7.15)	1,135 (8.09)	1,300 (8.29)

Table 3. Association of the Palaeolithic diet score with T2D (n=70,991) and hypertension risks (n=46,075).

	Non-cases Number (%)	Cases Number (%)	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Model 3 HR (95% CI)
Incident T2D					
1-SD increase	n=67,699	n=3,292	0.97 (0.93, 1.00)	0.99 (0.95, 1.02)	0.96 (0.93, 1.00)
Q1	12,454 (18.40)	621 (18.86)	Reference	Reference	Reference
Q2	16,900 (24.96)	789 (23.97)	0.89 (0.81, 0.99)	0.92 (0.83, 1.03)	0.91 (0.82, 1.02)
Q3	10,043 (14.83)	465 (14.13)	0.86 (0.76, 0.97)	0.88 (0.78, 0.99)	0.87 (0.77, 0.98)
Q4	13,359 (19.73)	675 (20.50)	0.91 (0.82, 1.02)	0.95 (0.85, 1.06)	0.94 (0.84, 1.05)
Q5	14,943 (22.07)	742 (22.54)	0.88 (0.79, 0.98)	0.93 (0.84, 1.04)	0.88 (0.79, 0.98)
P-trend			0.0825	<0.0001	<0.0001
Incident hypertension					
1-SD increase	n=33,571	n=12,504	0.98 (0.96, 1.00]	0.98 (0.96, 1.00)	0.97 (0.95, 0.99)
Q1	6,127 (18.25)	2,317 (18.46)	Reference	Reference	Reference
Q2	8,374 (24.94)	3,121 (24.87)	0.97 (0.92, 1.02)	0.97 (0.92, 1.02)	0.98 (0.92, 1.03)
Q3	4,997 (14.88)	1,817 (14.48)	0.94 (0.88, 1.00)	0.94 (0.88, 1.00)	0.94 (0.88, 1.00)
Q4	6,613 (19.70)	2,509 (19.99)	0.96 (0.91, 1.02)	0.95 (0.90, 1.01)	0.95 (0.89, 1.00)
Q5	7,460 (22.22)	2,786 (22.20)	0.94 (0.89, 0.99)	0.93 (0.88, 0.99)	0.91 (0.86, 0.96)
P-trend			0.029	0.019	0.0001

T2D:
Model 1: Adjusted for age (as the time-scale)
Model 2: Model 1 + family history of diabetes, educational level, hypercholesterolemia, hypertension and energy intake
Model 3: Model 2 + smoking status, physical activity and BMI

Hypertension:
Model 1: Adjusted for age (as the time-scale)
Model 2: Model 1 + family history of hypertension, educational level, hypercholesterolemia, diabetes and energy intake
Model 3: Model 2 + smoking status, physical activity and BMI

Table 4. Association of the combined Palaeolithic diet and lifestyle factors score with T2D (n=70,991) and hypertension risks (n=46,075).

	Non-cases Number (%)	Cases Number (%)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Incident T2D				
1-SD increase	n=67,699	n=3,292	0.80 (0.77, 0.83)	0.81 (0.79, 0.84)
Q1	14,445 (21.34)	880 (26.73)	Reference	Reference
Q2	11,847 (17.50)	627 (19.05)	0.83 (0.75, 0.92)	0.85 (0.77, 0.94)
Q3	13,902 (20.54)	660 (20.05)	0.72 (0.65, 0.80)	0.73 (0.66, 0.81)
Q4	12,756 (18.84)	564 (17.13)	0.66 (0.59, 0.73)	0.68 (0.61, 0.76)
Q5	14,749 (21.79)	561 (17.04)	0.55 (0.50, 0.61)	0.58 (0.52, 0.65)
P trend			<0.0001	<0.0001
Incident hypertension				
1-SD increase	n=33,571	n=12504	0.94 (0.92, 0.96)	0.94 (0.92, 0.95)
Q1	6,978 (20.79)	2,746 (21.88)	Reference	Reference
Q2	5,872 (17.49)	2,251 (17.94)	0.96 (0.91, 1.01)	0.95 (0.90, 1.00)
Q3	6,711 (19.99)	2,553 (20.34)	0.94 (0.89, 0.99)	0.93 (0.88, 0.98)
Q4	6,431 (19.16)	2,311 (18.41)	0.89 (0.84, 0.94)	0.87 (0.82, 0.92)
Q5	7,579 (22.58)	2,689 (21.43)	0.86 (0.82, 0.91)	0.85 (0.80, 0.90)
P trend			<0.0001	<0.0001

T2D:
Model 1: Adjusted for age (as the time-scale)
Model 2: Model 1 + family history of diabetes, educational level, hypercholesterolemia, hypertension and energy intake

Hypertension:
Model 1: Adjusted for age (as the time-scale)
Model 2: Model 1 + family history of hypertension, educational level, hypercholesterolemia, diabetes and energy intake