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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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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'Éducation Nationale

HDL- High-density lipoproteins

HR- Hazard ratio

LDL- Low-density lipoproteins

MGEN- Mutuelle Générale de l'Éducation 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 the 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 \geq 2,000 m), the average number
135 of flights of stairs climbed daily (0, 1-4, and \geq 5), the average amount of time spent weekly
136 doing light household activity (0, 1-4, 5-13, and \geq 14 hours) and heavy household activity (0,
137 1-4, and \geq 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 (meters²). 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 five 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”

¹All 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.

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

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

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

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

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

⁷Grains 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 (%)						
< Baccaulaureate	7,879 (11.09)	1,523 (11.65)	1,954 (11.05)	1,136 (10.81)	1,532 (10.92)	1,734 (11.06)
Baccaulaureate and Baccaulaureate +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)
> Baccaulaureate and Baccaulaureate +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).

			Model 1	Model 2	Model 3
	Non-cases Number (%)	Cases Number (%)	HR (95% CI)	HR (95% CI)	HR (95% CI)
<i>Incident T2D</i>					
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)
<i>P-trend</i>			0.0825	<0.0001	<0.0001
<i>Incident hypertension</i>					
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)
<i>P-trend</i>			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).

			Model 1	Model 2
	Non-cases Number (%)	Cases Number (%)	HR (95% CI)	HR (95% CI)
<i>Incident T2D</i>				
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)
<i>P trend</i>			<0.0001	<0.0001
<i>Incident hypertension</i>				
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)
<i>P trend</i>			<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				