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

Sanam Shah, Conor-James Macdonald, Douae El Fatouhi, Yahya Mahamat-Saleh, Francesca Mancini, Guy Fagherazzi, Gianluca Severi, Marie-Christine Boutron- Ruault, Nasser Laouali

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Sanam Shah,[1]; Conor-James MacDonald,[1]; Douae El Fatouhi,[1]; Yahya Mahamat-Saleh,[1]; Francesca Romana Mancini[1]; Guy Fagherazzi[2]; Gianluca Severi,[1,3]; Marie-Christine Boutron-Ruault,[1]; Nasser Laouali,[1]

Affiliations

[1]Paris-Saclay University, UVSQ, Univ. Paris-Sud, Inserm, Gustave Roussy, "Exposome and Heredity" team, CESP, F-94805, Villejuif, France

[2] Deep Digital Phenotyping Research Unit, Department of Population Health, Luxembourg Institute of Health (LIH), Strassen, Luxembourg

[3]Department of Statistics, Computer Science and Applications "G. Parenti", University of Florence, Italy

Corresponding Author

Gianluca Severi

Inserm U1018, "Exposome, Heredity, Cancer, and Health" Team, Gustave Roussy, 114 rue Edouard Vaillant, F-94805 Villejuif Cedex, France; Tel: +33 1 4211 4148; Fax: +33 1 4211 4148; Email: gianluca.severi@gustaveroussy.fr

Declarations:

Funding: This work was supported by a grant for the Nutriperso Project (IDEX Paris Saclay). This research was carried out using data from Inserm's E3N cohort with the support of the MGEN, the Institut GUSTAVE ROUSSY and the Ligue contre le Cancer for the constitution

and maintenance of the E3N cohort. This work has also benefited from State aid managed by the National Research Agency under the program "Investissement d'avenir" under the reference ANR-10-COHO-0006 as well as a subsidy from the "Ministère de l'enseignement supérieur, de la recherche et de l'innovation" for public service charges under the reference n°2102 918823.

Conflicts of interest/Competing interests: The authors declare that there is no duality of interest associated with this manuscript.

Ethics approval: The study received ethical approval from the French National Commission for Data Protection and Privacy (Commission Nationale Informatique et Libertés). The protocol is registered at clinicaltrials.gov as NCT03285230.

Availability of data and material: The datasets generated during and/or analysed for the current study are not publicly available, but are available from the corresponding author on reasonable request.

Consent to participate: All participants signed informed consent in compliance with the rules of the French National Commission for Computed Data and Individual Freedom (Commission Nationale Informatique et Libertés).

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.

Acknowledgements: The research was carried out using data from Inserm (French National Institutes for Health and Medical Research) E3N cohort, which was established and maintained with the support of the Mutuelle Générale de l'Education Nationale (MGEN), Gustave Roussy, and the French League against Cancer (LNCC). E3N-E4N is also supported by the French National Research Agency (ANR) under the Investment for the Future Program (PIA) (ANR-10-COHO-0006) and by the French Ministry of Higher Education, Research and Innovation (subsidy for public service charges #2102 918823). The authors are indebted to all participants for their continued participation. They are also grateful to all members of the E3N study group and contribution by Emmanuelle Correia for insights on the dietary data.

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

Abstract

Purpose: Patterns of change from the traditional Palaeolithic lifestyle to the modern lifestyle may partly explain the epidemic proportions of non-communicable diseases (NCDs). We investigated to what extent adherence to the Palaeolithic diet (PD) and the Palaeolithic-like lifestyle was associated with type 2 diabetes (T2D) and hypertension risks.

Methods: A study of 70,991 women from the E3N (*Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale*) cohort, followed up for nearly 20 years. There were 3,292 incident T2D and 12,504 incident hypertension cases that were validated. Dietary data were collected at baseline in 1993 via a food frequency questionnaire. The PD score and the Palaeolithic-like lifestyle score (PD, physical activity, smoking status and body mass index [BMI]) were derived and considered in quintiles. Multivariable Cox regression models were employed to estimate hazard ratios (HR) and 95% confidence intervals (CI) for incident T2D and hypertension.

Results: In the fully adjusted models, a 1-SD increase of the PD score was associated with 4% and 3% lower risks of T2D and hypertension, respectively. Those in the highest versus the lowest quintile of the score had HR (95% CI) of 0.88 (0.79, 0.98) and 0.91 (0.86, 0.96) for T2D and hypertension, respectively (*P*-trend <0.0001). Associations were stronger for the Palaeolithic-like lifestyle score; in the fully adjusted model a 1-SD increase of the score was associated with 19% and 6% lower risks of T2D and hypertension, respectively. Risks lowered successively with each increase in quintile; those in the highest versus the lowest quintile had HR (95% CI) of 0.58 (0.52, 0.65) and 0.85 (0.80, 0.90) for T2D and hypertension, respectively (*P*-trend < 0.0001).

Conclusions: Our data suggest that adhering to a PD based on fruits, vegetables, lean meats, fish, and nuts, and incorporating a Palaeolithic-like lifestyle could be promising options to prevent T2D and hypertension.

Keywords: Dietary pattern, The Palaeolithic diet, Palaeolithic-like lifestyle, Type 2 diabetes, Hypertension, Prospective study

Introduction

Over the last two to three decades, non-communicable diseases (NCDs) such as type 2 diabetes (T2D) and hypertension have become the most prevalent and costly health conditions in the developed world [1, 2]. According to the International Diabetes Federation, the prevalence of T2D is on the rise; from 415 million adults in 2015 worldwide, it is expected to increase to 700 million adults by 2045 [3]. Furthermore, the World Health Organization (WHO) has estimated the prevalence of hypertension to rise 29% by 2025 from a current prevalence of 972 million people which constitutes roughly 30% of the World's population [3–5]. These NCDs are strongly linked with cardiovascular diseases, the leading causes of morbidity and mortality globally [4]. Whilst the worldwide aging phenomenon is the main driver of the development of NCDs [6, 7], literature indicates the concurrent occurrence of changes in diet and lifestyle [6]. Hence, it is speculated that a shift back to the traditional lifestyle could avert this epidemic [8].

Studies have attributed lifestyle-related chronic diseases to modern behaviours which include increased sedentary time, smoking, and behaviours leading to excess energy intake and adiposity [9]. Furthermore, the Western diet, rich in processed meat, high-fat dairy, and refined products, is associated with increased production of oxygen species and oxidative stress which leads to T2D and hypertension [10, 11]. In contrast, traditional lifestyle patterns that resemble the culture of Palaeolithic people characterised by high levels of physical activity, energy balance which results in lean body mass, non-smoking [12, 13], and diets metabolically more attuned to traditional patterns of human evolution composed of lean meat, fish, fruit, vegetables, and nuts, with exclusion of processed foods, dairy products, and refined cereal products, the 'Palaeolithic-like diets' [14–16], could potentially prevent or reverse these disorders.

Palaeolithic nutrition has emerged as one of the most popular dietary patterns worldwide with few prospective studies to date. Whalen et al. constructed a Palaeolithic diet (PD) score as discordance between diet during the Palaeolithic period and that during the present era [17, 18]. Because the cumulative association between adverse diet and lifestyle factors, this score has been studied alone or in combination with lifestyle factors in relation to incident colorectal adenomas [17] and mortality [18] with findings of inverse associations. To be best of our knowledge, no study has examined the potential associations of long-term adherence to the PD with T2D and hypertension risks. Although the concept of the Palaeolithic nutrition is not novel, more studies with larger sample size and longer follow-up period were required to identify the PD as a healthier dietary pattern and an alternative that could be incorporated in future nutritional guidelines for prevention of NCDs.

Therefore, we aimed to investigate the extent of the influence of the PD on T2D and hypertension risks in the French E3N prospective cohort study of women. In addition, we aimed to clarify whether a higher adherence to an overall Palaeolithic-like lifestyle would contribute to benefits over and above those of the PD alone.

Materials and Methods

E3N cohort and Study Population for Analysis

The E3N (*Etude Epidémiologique auprès de femme de la Mutuelle Générale de l'Education Nationale*) is a large-scale French prospective cohort study initiated in 1990. It comprised of 98,995 women, aged 40-65 years at baseline and affiliated with the French national health insurance plan (*Mutuelle Générale de l'Education Nationale*, MGEN) for personnel of the French education system and their families. Participants were enrolled after informed written consent. Data were collected at inclusion and every two to three years by self-administered questionnaires (average response rate per questionnaire of approximately 83%). Detailed

information about health conditions, lifestyle, diet, treatments, etc. were recorded. Furthermore, all outpatient reimbursements for health expenditure since January 1, 2004 for each participant were obtained through the health insurance plan. These data included brand names, doses, and dates of drug reimbursements. The study received ethical approval from the French National Commission for Data Protection and Privacy (Commission Nationale Informatique et Libertés). The protocol is registered at clinicaltrials.gov as NCT03285230

Eligible participants (n = 74,522) completed and returned the baseline dietary questionnaire sent in 1993. The 1993 data served as the baseline and the cohort follow-up ended in 2014 (latest date of T2D and hypertension case validation in the E3N cohort). For the current study, we used data from two sub-samples (Supplementary Figure 1). First, we excluded the following participants: women with extreme energy intakes (i.e. below the 1st and above the 99th percentiles of the energy intake over energy requirement distribution in the population) (n = 1,774) and women who did not complete any follow-up questionnaire after the dietary questionnaire (n = 1,216). Then, for each outcome of interest, all prevalent cases (n = 824 and n = 28,164 for T2D and hypertension, respectively), i.e. cases reported to have occurred before the baseline date for the present study, i.e. the date when the 1993 questionnaire was answered, were excluded. This resulted in a final study size of 70,991 and 46,075 participants for T2D and hypertension analyses, respectively.

The Palaeolithic diet score

Dietary data were collected through a validated [19, 20] self-administered food frequency questionnaire with quantitative questions about consumption frequencies for eight eating moments from breakfast to after dinner snacks, portion sizes facilitated by photographs, and qualitative questions concerning specific food and drink items structured according to French 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

activity (0, 1-4, 5-13, and ≥14 hours) and vigorous recreational activity (0, 1-4,and ≥5 hours). A total physical activity score was calculated by multiplying the metabolic equivalent of task (MET-hours/week) of each activity by their frequency and duration. For smoking status, participants were classified smoker, ex-smoker and non-smoker. Self-reported height and weight at baseline were used to calculate BMI, defined as weight (kilograms) divided by squared height (meters^2). In the E3N cohort, self-reported anthropometry is considered reliable from a validation study [23]. In line with an existing 'evolutionary-concordance lifestyle score' developed by Cheng et al., which reflected the overall 'Palaeolithic diet and lifestyle score', we combined BMI, physical activity, smoking status, and the PD score to construct the 'Palaeolithic-like lifestyle score' [24]. Briefly, participants were categorised according to the quintile and tertile distributions of the PD score and lifestyle factors (except smoking status), respectively. For the lower quintile group of the PD corresponding to the lower adherence, value of 1 was assigned and one additional point was given for each increasing quintile. For physical activity, participants in the highest tertile were given values of 5 whereas values of 3 and 1 were given for each decreasing tertile, respectively; in contrast, the scoring was reversed for BMI. For smoking status, value of 5 was assigned to non-smokers and values of 3 and 1 for ex-smoker and smokers, respectively. Finally, all diet and lifestyle values for each participant were summed to reflect adherence to the Palaeolithic-like lifestyle score. In our cohort, the final score ranged from 17 to 85 and higher scores indicated better adherence.

Outcome ascertainment

Incident T2D was ascertained through self-reports, validation questionnaires and drug reimbursement files from 1993 until last validation of cases in 2014. The detailed procedure has been described elsewhere [25]. Before 2004, all potential cases of T2D were identified

through follow-up questionnaires that included questions on the diagnosis of T2D, diabetes-specific diets, diabetes drugs and hospitalisations for T2D. All potential cases were then contacted and asked to respond to a diabetes-specific questionnaire that included questions on the circumstances of diagnosis (year of diagnosis, symptoms, biological examinations, and fasting or random glucose concentration at diagnosis), T2D therapy (prescription of diet or physical activity, list of all glucose-lowering drugs already used), and the most recent concentrations of fasting glucose and HBA1c. The validation was based on WHO criteria as follows: fasting glucose of 7.0 mmol/L (126 mg/dL) or random glucose of 11.1 mmol/L (200 mg/dL)] and/or reported diabetic drugs, and/or last values of fasting glucose or glycated haemoglobin concentrations reported to be 7.0 mmol/L (126 mg/dL) or 7%, respectively. After 2004, T2D cases were identified through a drug reimbursement database. All women with at least two reimbursements for any glucose-lowering medication within a 1-year period were considered to be validated T2D cases, with the date of diagnosis defined as the date of their first reimbursement.

For hypertension, participants were asked to report whether they had hypertension at baseline (1993) and in each follow-up questionnaire (1995, 1997, 2000, 2002, 2005, 2008, 2011, and 2014), the date of diagnosis, and the use of antihypertensive treatments. The month and year of diagnosis were provided for most cases (69%). For individuals who were missing the month of diagnosis (14% of cases), it was imputed to June of the year of diagnosis. The median time between the date of diagnosis and the date of response to the first questionnaire after diagnosis was 12 months. Thus, for cases with no year of diagnosis (n = 17%), we assigned it to be 12 months before they reported hypertension in a questionnaire. From 2004, a drug reimbursement database became available for 97.6 % of participants. We used the self-reported date of diagnosis or the first date of drug reimbursement for antihypertensive medications (Anatomical Therapeutic Chemical Classification System codes C02, C03, C07,

C08, and C09) whatever happened first, as the date of diagnosis for cases identified after 2004. In addition, using the information of the MGEN health insurance plan drug claim database, we assessed the validity of self-reported hypertension within the E3N cohort. We compared hypertension self-report to antihypertensive drug reimbursement (any of the above specified codes). A positive predictive value of 82% was observed among women alive in January 2004 and followed up to their response to the last considered questionnaire in 2008 [26].

Covariates

Potential confounders of the association between the PD and T2D and hypertension risks were accounted for after literature review of their associations with both exposure and outcomes. They consisted of baseline self-reported sociodemographic and anthropometric characteristics, total energy intake, physical activity, smoking status, BMI, personal history of hypercholesterolemia, and family history of diabetes and hypertension.

Statistical Analyses

Description of participants' baseline characteristics according to T2D and hypertension status across quintiles of the PD and the combined PD and lifestyle scores were described using mean (standard deviation [SD]) for continuous variables, and frequencies and proportions for categorical variables. Cox proportional hazards regression models with age as the time-scale were used to estimate hazards ratios (HR) and 95% confidence intervals (CI). Participants were followed from age at baseline until age at diagnosis of T2D or hypertension, or age at death, or age at last follow-up, or age at the end of the follow-up period (2014 for T2D and hypertension), whichever occurred first. The proportional hazards assumption was tested graphically using Schoenfeld Residuals. No major violations were observed.

All scores were modelled in three ways. First, as continuous variable where we reported estimates for a 1-SD increase assuming a linear association between the scores and incidence of health events. Second, to check this assumption we used multivariate restricted cubic splines with fives knots placed at the 5th, 27.5th, 50th, 72.5th and 95th percentiles of each score to provide a graphical representation [27]. Splines allowed us to test whether there was a significant departure from a linear association. Finally, all scores were categorised into quintiles, and the first quintile group was considered as the reference category to assess whether subsequently higher categories were associated with lower risks of T2D and hypertension.

Main models of associations between the PD and risks of T2D and hypertension were adjusted for the following potential confounders: age (as the time-scale), educational level (< Baccalaureate, Baccalaureate to Baccalaureate +2, > Baccalaureate +2), smoking status (non-smoker, past, current), BMI (continuous), physical activity (continuous), family history of T2D (yes, no [T2D model]), family history of hypertension (yes, no [hypertension model]), personal history of hypercholesterolemia (yes, no) and energy intake (continuous). The main models for the associations between the combined score and T2D and hypertension risks were not adjusted for BMI, smoking status and physical activity since these variables were used to build the combined score. Missing values were less than 5% for all variables and were either assigned to the median for continuous variables or the mode for categorical variables.

Sensitivity analyses

Two sensitivity analyses were performed. First, our main analyses were repeated after excluding women with T2D and hypertension diagnosed in the first 5 years of follow-up to

examine potential reverse causation. Finally, we further adjusted the main models for adherence to the Western dietary pattern [28].

All analyses were conducted using SAS statistical software, version 9.4 (SAS Institute, Inc., Cary, North Carolina).

Results

Baseline characteristics

Over a mean of 18.81 (SD 4.3) years of follow-up, 3,292 (4.64%) and 12,504 (27.14%) validated incident T2D and hypertension cases were identified, respectively. Table 2 and Supplementary Tables 1 to 3 present the baseline characteristics of the study, overall and according to quintiles of the PD and the combined score. Participants had a mean (SD) age of 53 (6.7) years at baseline. In general, higher adherence to the PD score and the combined PD and lifestyle score was associated with older age, higher physical activity, and increased frequencies of non-smoking and hypercholesterolemia at baseline.

The Palaeolithic diet score and type 2 diabetes and hypertension risks

Table 3 provides HRs of the associations between the PD, and T2D and hypertension risks. After controlling for all covariates in the fully adjusted Model 3, the HR (95% CI) per SD increase for T2D was 0.96 (0.93, 1.00). Similarly, there was a modest reduction in the HR (95% CI) per SD increase for hypertension in Models 1 and 2, 0.98 (0.96, 1.00), which became slightly stronger in Model 3, 0.97 (0.95, 0.99). Spline variables analysis did not show departures from linear associations (P = 0.920 and P = 0.928 for T2D and hypertension, respectively (Supplementary Figure 2). Higher quintiles of the PD score were associated with lower risks of incident T2D (Model 3, P-trend <0.0001) and hypertension (Model 1: P-trend 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

Considering the growing popularity of the PD, we delineated its role in the prevention of NCDs. To the best of our knowledge, this study is the first to assess the association of adherence to the PD alone or combined with other lifestyle factors, with T2D and hypertension risks. Findings from this large-scale prospective cohort followed for approximately 20 years indicated inverse linear associations between adherence to the PD and incidences of both T2D and hypertension. Furthermore, adherence to the Palaeolithic-like lifestyle was strongly inversely associated with risks of both T2D and hypertension, independently of most known or potential risk factors or confounders. In comparison to the previously reported short term beneficial effects of adherence to the PD, such as favourable body weight and metabolic balance [15, 29–31], these findings provide evidence of potential long-term benefits on T2D and hypertension risks.

Prior studies on the PD have been conducted on high risk populations such as T2D patients and despite the heterogeneity in the PD definition, have reported benefits. When compared with the diabetic diet, the PD was associated with a larger reduction in HBA1c, triglycerides, blood pressure, and weight; however, no difference in reduction of fasting plasma glucose (FPG) were seen [29]. Furthermore, when compared with the usual diet, the PD was associated with lower plasma insulin, low-density lipoproteins (LDL), and triglycerides [15], but not associated with high-density lipoproteins (HDL) and FPG [32]. Last, in contrast to Nordic Nutritional Recommendations, there was no reduction in FPG and insulin levels [33]. The PD most closely resembles a low carbohydrate diet in composition [34]. Low carbohydrate diets with potentially higher protein and fat intake as compared to carbohydrates have been reported to be inversely associated with T2D risk [35] as well as associated with beneficial glucose control in T2D patients [36]. In addition, there is systolic and diastolic blood pressure reduction, weight loss, and improvement in lipid profile [37, 38]. 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.

Prior studies have proposed potential mechanisms for the beneficial associations of the PD. It has been hypothesized that insulin resistance emerged as an adaptation to low carbohydrate foods consumed during the Palaeolithic period [32]. Moreover, population differences, individual polymorphisms, and genetic variants promote insulin resistance which is aggravated by consumption of high glycaemic index foods [48]. Diet has undergone significant changes since the Palaeolithic period unlike the human genome, which has varied slightly [49, 50]. In this regard, the PD which is devoid of high glycaemic index foods, processed foods, and fats which increase extracellular acidity, could be potentially protective against inflammation. High intakes of micronutrients such as magnesium, vitamins C and E, carotenoids, and dietary fibre, and low intakes of saturated fats are protective as well [51]. Indeed animal experiments have found beneficial effects of the PD on weight, fat deposits, inflammatory markers, blood pressure, and insulin sensitivity [52]. Furthermore, the PD is marked by low sugar consumption, fruits and honey being the main sources of simple sugars. This elicits an improvement in the metabolic profile via increased HDL as a consequence of loss of fat, low glycaemic load, and higher levels of long chain fatty acids [53]. Lastly, the PD enhances satiety with subsequent low energy intake via incretin and anorectic gut hormones as well as by consumption of low energy-density foods such as fruits, vegetables, lean meat and fish, and water [54, 55].

As regards the association of the combined score of diet and lifestyle factors, each modifiable risk factor has been reported to exert an independent influence on the risk of disease [56–59]. Adiposity has demonstrated a strong association with incident diabetes, and physical activity, healthy diet, non-smoking, and moderate use of alcohol have been reported to have associations independent of adiposity [60]. Besides individual risk factors, each additional factor and a combination of different lifestyle factors which included physical activity,

limited alcohol intake, and a healthy diet have been associated with a significant reduction in risk of hypertension as well [47].

Our study has the following strengths: first, we derived the PD score and the Palaeolithic-like lifestyle score to investigate their associations in relation to two major NCDs, T2D and hypertension. Such scoring reflects the overall dietary and lifestyle patterns and the risks of diseases and is more intuitive in understanding associations. Second, the prospective nature of our study allowed assessment of NCDs incidence in a large sample with a long follow-up period. Last, validated cases of T2D and hypertension minimized bias related to recall and inclusion of false positive cases.

Limitations of our study merit further discussion. First, diet was assessed on one set of responses collected in 1993 therefore any changes in diet over the follow-up period were not accounted for. Second, the use of self-reported questionnaires may have led to non-differential misclassification of dietary exposure as reported in the literature [61]. Third, although the main potential confounding factors were accounted for, there could be residual confounding due to unadjusted lifestyle factors [62]. Last, the findings would need to be generalized with caution as the cohort consisted of women with presumably good levels of education and health awareness hence confirmation in other populations is required. However, assuming causality of findings, we would expect stronger protective associations of the PD and the Palaeolithic-like lifestyle based on the dietary and lifestyle habits of the general population.

Conclusions

In conclusion, our findings suggest that the PD and the Palaeolithic-like lifestyle patterns are associated with lower risks of T2D and hypertension. The PD based on fruit, vegetables, lean meat, fish, and nuts, with low intakes of dairy, grains, and salt, may have a favourable role in

the prevention of chronic diseases of the modern era. These findings also support existing guidelines of behavioural lifestyle interventions for the prevention or delay of non-communicable chronic diseases. Further studies are required to validate findings in other population groups and to delineate the precise mechanisms of the favourable associations 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).

			Model 1	Model 2	Model 3
	Non-cases Number (%)	Cases Number (%)	HR (95% CI)	HR (95% CI)	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).

			Model 1	Model 2
	Non-cases Number (%)	Cases Number (%)	HR (95% CI)	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