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.

Sanam Shah,¹; Conor-James MacDonald,¹; Douae El Fatouhi,¹; Yahya Mahamat-Saleh,¹; Francesca Romana Mancini¹; Guy Fagherazzi²; Gianluca Severi,^{1,3}; Marie-Christine Boutron-Ruault,¹; Nasser Laouali,¹

Affiliations

¹Paris-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

³Department 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'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

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

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.

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 the 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

months. All food intakes were converted into daily intakes of energy and nutrients by means of a study-specific Food Composition Database derived from the French Information Center on Food Quality [21] .

A score reflecting adherence to the PD was employed as described previously by Whalen et al. [17]. Briefly, all food items were categorised into 14 food groups. These food groups were classified as more characteristic of the PD (vegetables, fruits, fruit and vegetable diversity score, lean meat, fish, nuts, and calcium), and less characteristic of the PD (red and processed meat, dairy foods, sugar sweetened beverages, baked goods, grains and starches, sodium, and alcohol) (Table 1). Dietary diversity is a qualitative measurement of the spectrum of foods consumed and is a proxy indicator of nutrient adequacy [22]. In our study, the fruit and vegetable diversity score represented the number of components of the fruit and vegetable group reported to be consumed by each participant. For dietary scoring, intake of each food component was categorised as quintiles in accordance with the study population intake distributions. Values of 1 to 5 were assigned to each quintile with higher values given to higher intake of food components considered characteristic of the PD and lower values given to higher consumption of foods considered less characteristic of the PD. The possible PD score ranged from 14 to 70 and higher scores indicated higher levels of adherence to the PD.

The Palaeolithic-like lifestyle score (combined score)

Lifestyle factors including physical activity, smoking status, and BMI were obtained at baseline and follow-up questionnaires. Physical activity was assessed through six questions based on usual distance walked daily (<500, 500-2,000, and $\geq 2,000$ m), the average number of flights of stairs climbed daily (0, 1-4, and ≥ 5), the average amount of time spent weekly doing light household activity (0, 1-4, 5-13, and ≥ 14 hours) and heavy household activity (0, 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 (meters²). 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 five 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,

those in the highest quintile had a 12% lower risk [HR (95% CI): 0.88 (0.79; 0.98)]. For hypertension, after full adjustment, those in the highest quintile had a 9% lower risk [HR (95% CI): 0.91 (0.86, 0.96)].

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

Table 4 provides HRs of the associations between the PD score combined with lifestyle factors, and T2D and hypertension risks. The combined score was much more strongly inversely associated with T2D and hypertension risks in both Models 1 and 2. After controlling for all covariates in Model 2, there was a 19% lower risk of T2D and 6% lower risk of hypertension [HR (95% CI) per SD increase: 0.81 (0.79, 0.84) and 0.94 (0.92, 0.95), for T2D and hypertension, respectively]. There was no departure from a linear association [$P = 0.983$ and $P = 0.518$ for T2D and hypertension, respectively (Supplementary Figure 3)]. When the combined score was categorised into quintiles, we observed a gradual inverse association from quintile 1 to 5 across models for both outcomes (P -trend <0.0001). For T2D, in Model 2, those in the highest versus lowest quintile had a 42% lower risk [HR (95% CI): 0.58 (0.52, 0.65)]. The association was not as strong for hypertension risk with only a 15% lower risk for those in the highest versus lowest quintile [HR (95% CI): 0.85 (0.80, 0.90)].

Sensitivity analyses

Results were not substantially modified when participants with T2D and hypertension diagnosed in the first 5 years of follow-up ($n = 383$ and $n = 2910$ for T2D and hypertension, respectively) were excluded, and further adjustment made for the Western dietary pattern (data not tabulated).

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

negatively correlated to Western diet ($r = -0.42$; $P < 0.001$) and our findings are consistent with previous studies that examined these dietary patterns in relation with risks of T2D [39, 40] and hypertension [41–43].

In recent years, the role of combined lifestyle factors on the risk of NCDs has been emphasised and our findings concur with studies from France and the US. A study in the E3N cohort in France reported that one-third of T2D cases could be attributed to high adherence to a Western diet, and that a large proportion of cases could be avoided by adopting a healthy lifestyle alone [44]. Another study from the US assessed the impact of a modifiable lifestyle score consisting of five culturally appropriate lifestyle factors, namely physical activity, television watching, American Heart Association (AHA) healthy diet score, smoking, and sleep apnea syndrome; there was a 17% lower risk of T2D over eight years of follow-up [45]. Moreover, a systematic review reported an approximately 75% reduction in risk of T2D by adopting the healthiest lifestyle which comprised of a combination of multiple healthy lifestyle factors [46]. A similar concept has been applied in hypertension research. A study in the French population evaluated the impact of a combination of healthy lifestyle factors namely BMI, physical activity, alcohol consumption, and the Dietary Approach to Stop Hypertension (DASH) style diet score on the risk of hypertension, and reported a 65% reduced risk of hypertension [47]. Although there are empirical differences in the construction of various combinations of lifestyle scores and dietary patterns, we detected a trend towards inverse relationships between T2D and hypertension, and healthy lifestyle indices. Interestingly, when lifestyle factors were analysed individually, we observed weak associations with T2D and hypertension. The strongest associations were observed when combining these lifestyle factors, suggesting synergistic associations on T2D and 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

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.

References

1. American Diabetes Association (2018) Economic Costs of Diabetes in the U.S. in 2017. *Diabetes Care* 41:917–928. <https://care.diabetesjournals.org/content/41/5/917>. Accessed 10 Mar 2021
2. Kirkland EB, Heincelman M, Bishu KG, et al (2018) Trends in Healthcare Expenditures Among US Adults With Hypertension: National Estimates, 2003-2014. *J Am Heart Assoc* 7. <https://doi.org/10.1161/JAHA.118.008731>
3. International Diabetes Federation Diabetes Atlas (2019) Global diabetes data report 2010-2045. <https://diabetesatlas.org/data/en/world/>. Accessed 10 Mar 2021
4. World Health Organization (2013) A global brief on hypertension: silent killer, global public health crisis. https://apps.who.int/iris/bitstream/handle/10665/79059/WHO_DCO_WHD_2013.2_eng.pdf. Accessed 10 Mar 2021
5. Kopp W (2019) How Western Diet And Lifestyle Drive The Pandemic Of Obesity And Civilization Diseases. *Diabetes Metab Syndr Obes* 12:2221–2236. <https://doi.org/10.2147/DMSO.S216791>
6. Fabbri E, Zoli M, Gonzalez-Freire M, et al (2015) Aging and Multimorbidity: New Tasks, Priorities, and Frontiers for Integrated Gerontological and Clinical Research. *J Am Med Dir Assoc* 16:640–647. <https://doi.org/10.1016/j.jamda.2015.03.013>

7. Popkin BM, Adair LS, Ng SW (2012) Global nutrition transition and the pandemic of obesity in developing countries. *Nutr Rev* 70:3–21.

<https://doi.org/10.1111/j.1753-4887.2011.00456.x>
8. Pontzer H, Wood BM, Raichlen DA (2018) Hunter-gatherers as models in public health. *Obes Rev* 19 Suppl 1:24–35.

<https://doi.org/10.1111/obr.12785>
9. Egger G, Dixon J (2014) Beyond Obesity and Lifestyle: A Review of 21st Century Chronic Disease Determinants. *BioMed Research International* 2014:1–12.

<https://doi.org/10.1155/2014/731685>
10. González J, Valls N, Brito R, Rodrigo R (2014) Essential hypertension and oxidative stress: New insights. *World J Cardiol* 6:353–366.

<https://doi.org/10.4330/wjc.v6.i6.353>
11. Oguntibeju OO (2019) Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. *Int J Physiol Pathophysiol Pharmacol* 11:45–63.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6628012/pdf/ijppp0011-0045.pdf>
12. Aleksandrova K, Pischon T, Jenab M, et al (2014) Combined impact of healthy lifestyle factors on colorectal cancer: a large European cohort study. *BMC Med* 12:168.

<https://doi.org/10.1186/s12916-014-0168-4>
13. Hastert TA, White E (2016) Association between meeting the WCRF/AICR cancer prevention recommendations and colorectal cancer incidence: results from the VITAL cohort. *Cancer Causes Control* 27:1347–1359.

<https://doi.org/10.1007/s10552-016-0814-6>

14. Masharani U, Sherchan P, Schloetter M, et al (2015) Metabolic and physiologic effects from consuming a hunter-gatherer (Paleolithic)-type diet in type 2 diabetes. *Eur J Clin Nutr* 69:944–948.

<https://doi.org/10.1038/ejcn.2015.39>
15. Frassetto LA, Schloetter M, Mietus-Synder M, et al (2009) Metabolic and physiologic improvements from consuming a paleolithic, hunter-gatherer type diet. *Eur J Clin Nutr* 63:947–955.

<https://doi.org/10.1038/ejcn.2009.4>
16. Cordain L (2015) The Nutritional Characteristics of a Contemporary Diet Based Upon Paleolithic Food Groups.

<https://thepaleodiet.com/wp-content/uploads/2015/08/The-Nutritional-Characteristics-of-a-Contemporary-Diet-Based-Upon-Paleolithic-Food-Groups-The-Paleo-Diet.pdf> Accessed 10 Mar 2021
17. Whalen KA, McCullough M, Flanders WD, et al (2014) Paleolithic and Mediterranean diet pattern scores and risk of incident, sporadic colorectal adenomas. *Am J Epidemiol* 180:1088–1097.

<https://doi.org/10.1093/aje/kwu235>
18. Whalen KA, Judd S, McCullough ML, et al (2017) Paleolithic and Mediterranean Diet Pattern Scores Are Inversely Associated with All-Cause and Cause-Specific Mortality in Adults. *J Nutr* 147:612–620.

<https://doi.org/10.3945/jn.116.241919>

19. Lucas F, Niravong M, Villeminot S, et al (1995) Estimation of food portion size using photographs: validity, strengths, weaknesses and recommendations. *Journal of Human Nutrition and Dietetics* 8:65–74.
<https://doi.org/10.1111/j.1365-277X.1995.tb00296.x>
20. van Liere MJ, Lucas F, Clavel F, et al (1997) Relative validity and reproducibility of a French dietary history questionnaire. *Int J Epidemiol* 26 Suppl 1:S128–36.
https://doi.org/10.1093/ije/26.suppl_1.s128.
21. Ciquel Table de composition nutritionnelle des aliments.
<https://ciquel.anses.fr>. Accessed 10 Mar 2021
22. Food And Agriculture Organization Of The United Nations (2013) Guidelines for measuring household and individual dietary diversity. <http://www.fao.org/3/i1983e/i1983e.pdf>.
Accessed 10 Mar 2021
23. Tehard B, van Liere MJ, Com Noug C, Clavel-Chapelon F (2002) Anthropometric measurements and body silhouette of women: validity and perception. *J Am Diet Assoc* 102:1779–1784.
[https://doi.org/10.1016/s0002-8223\(02\)90381-0](https://doi.org/10.1016/s0002-8223(02)90381-0)
24. Cheng E, Um CY, Prizment AE, et al (2018) Evolutionary-Concordance Lifestyle and Diet and Mediterranean Diet Pattern Scores and Risk of Incident Colorectal Cancer in Iowa Women. *Cancer Epidemiol Biomarkers Prev* 27:1195–1202.
<https://doi.org/10.1158/1055-9965.EPI-17-1184>
25. Mancini FR, Affret A, Dow C, et al (2018) Dietary antioxidant capacity and risk of type 2 diabetes in the large prospective E3N-EPIC cohort. *Diabetologia* 61:308–316.
<https://doi.org/10.1007/s10654-019-00548-9>

26. Lajous M, Rossignol E, Fagherazzi G, et al (2016) Flavonoid intake and incident hypertension in women. *Am J Clin Nutr* 103:1091–1098.

<https://doi.org/10.3945/ajcn.115.109249>
27. Desquilbet L, Mariotti F (2010) Dose-response analyses using restricted cubic spline functions in public health research. *Stat Med* 29:1037–1057.

<https://doi.org/10.1002/sim.3841>
28. Cottet V, Touvier M, Fournier A, et al (2009) Postmenopausal breast cancer risk and dietary patterns in the E3N-EPIC prospective cohort study. *Am J Epidemiol* 170:1257–1267.

<https://doi.org/10.1093/aje/kwp257>
29. Jönsson T, Granfeldt Y, Åhrén B, et al (2009) Beneficial effects of a Paleolithic diet on cardiovascular risk factors in type 2 diabetes: a randomized cross-over pilot study. *Cardiovasc Diabetol* 8:35.

<https://doi.org/10.1186/1475-2840-8-35>
30. Lindeberg S, Jönsson T, Granfeldt Y, et al (2007) A Palaeolithic diet improves glucose tolerance more than a Mediterranean-like diet in individuals with ischaemic heart disease. *Diabetologia* 50:1795–1807.

<https://doi.org/10.1007/s00125-007-0716-y>
31. Osterdahl M, Koçturk T, Koochek A, Wändell PE (2008) Effects of a short-term intervention with a paleolithic diet in healthy volunteers. *Eur J Clin Nutr* 62:682–685.

<https://doi.org/10.1038/sj.ejcn.1602790>

32. Manheimer EW, van Zuuren EJ, Fedorowicz Z, Pijl H (2015) Paleolithic nutrition for metabolic syndrome: systematic review and meta-analysis. *Am J Clin Nutr* 102:922–932.

<https://doi.org/10.3945/ajcn.115.113613>
33. Mellberg C, Sandberg S, Ryberg M, et al (2014) Long-term effects of a Palaeolithic-type diet in obese postmenopausal women: a 2-year randomized trial. *Eur J Clin Nutr* 68:350–357.

<https://doi.org/10.1038/ejcn.2013.290>
34. Gupta L, Khandelwal D, Lal PR, et al (2019) Palaeolithic Diet in Diabetes and Endocrinopathies - A Vegan's Perspective. *Eur Endocrinol* 15:77–82.

<https://doi.org/10.17925/EE.2019.15.2.77>
35. Halton TL, Liu S, Manson JE, Hu FB (2008) Low-carbohydrate-diet score and risk of type 2 diabetes in women. *Am J Clin Nutr* 87:339–346.

<https://doi.org/10.1093/ajcn/87.2.339>
36. Meng Y, Bai H, Wang S, et al (2017) Efficacy of low carbohydrate diet for type 2 diabetes mellitus management: A systematic review and meta-analysis of randomized controlled trials. *Diabetes Res Clin Pract* 131:124–131.

<https://doi.org/10.1016/j.diabres.2017.07.006>
37. Unwin DJ, Tobin SD, Murray SW, et al (2019) Substantial and Sustained Improvements in Blood Pressure, Weight and Lipid Profiles from a Carbohydrate Restricted Diet: An Observational Study of Insulin Resistant Patients in Primary Care. *Int J Environ Res Public Health* 16.: <https://doi.org/10.3390/ijerph16152680>.

<https://doi.org/10.3390/ijerph16152680>

38. Dong T, Guo M, Zhang P, et al (2020) The effects of low-carbohydrate diets on cardiovascular risk factors: A meta-analysis. *PLoS One* 15:e0225348.

<https://doi.org/10.1371/journal.pone.0225348>
39. Schwingshackl L, Missbach B, König J, Hoffmann G (2015) Adherence to a Mediterranean diet and risk of diabetes: a systematic review and meta-analysis. *Public Health Nutr* 18:1292–1299.

<https://doi.org/10.1017/s1368980014001542>
40. Cordain L, Eaton SB, Sebastian A, et al (2005) Origins and evolution of the Western diet: health implications for the 21st century. *Am J Clin Nutr* 81:341–354.

<https://doi.org/10.1093/ajcn.81.2.341>
41. De Pergola G, D'Alessandro A (2018) Influence of Mediterranean Diet on Blood Pressure. *Nutrients* 10(11):1700.

<https://doi.org/10.3390/nu10111700>
42. Monge A, Lajous M, Ortiz-Panozo E, et al (2018) Western and Modern Mexican dietary patterns are directly associated with incident hypertension in Mexican women: a prospective follow-up study. *Nutr J* 17:21.

<https://doi.org/10.1186/s12937-018-0332-3>
43. Sun J, Buys NJ, Hills AP (2014) Dietary pattern and its association with the prevalence of obesity, hypertension and other cardiovascular risk factors among Chinese older adults. *Int J Environ Res Public Health* 11:3956–3971.

<https://doi.org/10.3390/ijerph110403956>

44. Rajaobelina K, Dow C, Romana Mancini F, et al (2019) Population attributable fractions of the main type 2 diabetes mellitus risk factors in women: Findings from the French E3N cohort. *J Diabetes* 11:242–253.

<https://doi.org/10.1111/1753-0407.12839>
45. Joseph JJ, Echouffo-Tcheugui JB, Talegawkar SA, et al (2017) Modifiable Lifestyle Risk Factors and Incident Diabetes in African Americans. *Am J Prev Med* 53:e165–e174.

<https://doi.org/10.1016/j.amepre.2017.06.018>
46. Zhang Y, Pan X-F, Chen J, et al (2020) Combined lifestyle factors and risk of incident type 2 diabetes and prognosis among individuals with type 2 diabetes: a systematic review and meta-analysis of prospective cohort studies. *Diabetologia* 63:21–33.

<https://doi.org/10.1007/s00125-019-04985-9>
47. Lelong H, Kesse-guyot E, Galan P, et al (2017) Combination of healthy lifestyle factors on the risk of hypertension in a large cohort of healthy adults. *Journal of Hypertension* 11(7): 1687.

<https://doi.org/10.3390/nu11071687>
48. Cochran G, Harpending H (2009) *The 10,000 Year Explosion: How Civilization Accelerated Human Evolution*. Basic Books.
49. Pritchard JK (2010). *How We Are Evolving*. Scientific American.

<https://www.scientificamerican.com/article/how-we-are-evolving>. Accessed 10 Mar 2021
50. Klonoff DC (2009) The beneficial effects of a Paleolithic diet on type 2 diabetes and other risk factors for cardiovascular disease. *J Diabetes Sci Technol* 3:1229–1232.

<https://doi.org/10.1177/1932296809000300601>

51. Simopoulos AP (2008) The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med* 233:674–688.

<https://doi.org/10.3181/0711-MR-311>
52. Jönsson T, Åhrén B, Pacini G, et al (2006) A Paleolithic diet confers higher insulin sensitivity, lower C-reactive protein and lower blood pressure than a cereal-based diet in domestic pigs. *Nutr Metab* 3:39.

<https://doi.org/10.1186/1743-7075-3-39>
53. Silva Figueiredo P, Carla Inada A, Marcelino G, et al (2017) Fatty Acids Consumption: The Role Metabolic Aspects Involved in Obesity and Its Associated Disorders. *Nutrients* 9.:

<https://doi.org/10.3390/nu9101158>.

<https://doi.org/10.3390/nu9101158>
54. Jönsson T, Granfeldt Y, Lindeberg S, Hallberg A-C (2013) Subjective satiety and other experiences of a Paleolithic diet compared to a diabetes diet in patients with type 2 diabetes. *Nutr J* 12:105.

<https://doi.org/10.1186/1475-2891-12-105>
55. Genoni A, Lyons-Wall P, Lo J, Devine A (2016) Cardiovascular, Metabolic Effects and Dietary Composition of Ad-Libitum Paleolithic vs. Australian Guide to Healthy Eating Diets: A 4-Week Randomised Trial. *Nutrients* 8(5):314.

<https://doi.org/10.3390/nu805031456>.
56. Huang Z, Willett WC, Manson JE, et al (1998) Body weight, weight change, and risk for hypertension in women. *Ann Intern Med* 128:81–88.

<https://doi.org/10.7326/0003-4819-128-2-199801150-00001>

57. Pereira MA, Folsom AR, McGovern PG, et al (1999) Physical activity and incident hypertension in black and white adults: the Atherosclerosis Risk in Communities Study. *Prev Med* 28:304–312.

<https://doi.org/10.1006/pmed.1998.0431>
58. Parker ED, Schmitz KH, Jacobs DR Jr, et al (2007) Physical activity in young adults and incident hypertension over 15 years of follow-up: the CARDIA study. *Am J Public Health* 97:703–709.

<https://doi.org/10.2105/AJPH.2004.055889>
59. Bellou V, Belbasis L, Tzoulaki I, Evangelou E (2018) Risk factors for type 2 diabetes mellitus: An exposure-wide umbrella review of meta-analyses. *PLoS One* 13:e0194127.

<https://doi.org/10.1371/journal.pone.0194127>
60. Reis JP, Loria CM, Sorlie PD, et al (2011) Lifestyle factors and risk for new-onset diabetes: a population-based cohort study. *Ann Intern Med* 155:292–299.

<https://doi.org/10.7326/0003-4819-155-5-201109060-00006>
61. Naska A, Lagiou A, Lagiou P (2017) Dietary assessment methods in epidemiological research: current state of the art and future prospects. *F1000Res* 6:926.

<https://doi.org/10.12688/f1000research.10703.1>
62. Lee JA, Sunwoo S, Kim YS, et al (2016) The Effect of Sleep Quality on the Development of Type 2 Diabetes in Primary Care Patients. *J Korean Med Sci* 31:240–246.

<https://doi.org/10.3346/jkms.2016.31.2.240>

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).

	Non-cases Number (%)	Cases Number (%)	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Model 3 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
<p>T2D:</p> <p>Model 1: Adjusted for age (as the time-scale)</p> <p>Model 2: Model 1 + family history of diabetes, educational level, hypercholesterolemia, hypertension and energy intake</p> <p>Model 3: Model 2 + smoking status, physical activity and BMI</p> <p>Hypertension:</p> <p>Model 1: Adjusted for age (as the time-scale)</p> <p>Model 2: Model 1 + family history of hypertension, educational level, hypercholesterolemia, diabetes and energy intake</p> <p>Model 3: Model 2 + smoking status, physical activity and BMI</p>					

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)
<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
<p>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</p> <p>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</p>				