Statistical Analysis Plan (SAP)

Risk of hip fracture in meat-eaters, pescatarians, and vegetarians in the UK Biobank

Version 1.0

Version date: 21st September, 2022

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Contents

Abbreviations

NHS	National Health Service
WHO	World Health Organisation
ICD	International Classification of Disease
HES	Hospital Episode Statistics
SMR	Scottish Morbidity Record
PEDW	Patient Episode Database for Wales
HR	Hazard ratio
SD	Standard deviation
BMI	Body mass index
BMD	Bone mineral density
BMC	Bone mineral content
DXA	Dual-energy X-ray absorptiometry
IGF-1	Insulin-like growth factor 1
AHEI	Alternative healthy eating index
FFQ	Food frequency questionnaire
IORW	Inverse odds ratio weighting
MET	Metabolic equivalent task
HRT	Hormone replacement therapy

1.0 Objectives

1.1 Primary objective

- To investigate the risk of hip fracture in occasional meat-eaters, pescatarians, and vegetarians compared to regular meat-eaters.

1.2 Secondary objectives

- To determine the roles of potential modifying factors on associations between each diet group and hip fracture risk, including age, sex, body mass index (BMI), and adherence to the Alternative Healthy Eating Index (AHEI).
- To investigate interactions between diet groups and genotypes on bone mineral density (BMD) and hip fracture risk.
- To determine the roles of potential mediating factors on any observed associations between diet groups and hip fracture risk, including the roles of BMI, measures of body composition measures, circulating levels of vitamin D and IGF-1, and dietary intake of protein, calcium, vitamin B12, and omega-3 fatty acids.

2.0 Study design

The dataset will use participant data from the pre-existing UK Biobank database. This is a prospective cohort of over 500,000 adults across England, Scotland, and Wales, aged 40 – 69 years at recruitment in 2006-2010. Participants were recruited via National Health Service (NHS) patient registers, and attended one of 22 assessment centres across the UK, where participants were asked to complete a touchscreen questionnaire, verbal interview, physical measures, and a biosample collection (more information available at UK Biobank - UK Biobank).

3.0 Data cleaning

3.1 Exclusion criteria

Participants will be excluded from all analyses for any of the following reasons:

- Unable to link dietary and lifestyle data with hospital data (e.g. no NHS number provided and unable to match with records).
- Had a hip fracture (World Health Organisation, WHO International Classification of Disease (ICD-10) codes S72.0-S72.2) or osteoporosis (ICD-10 codes M80-M82) before or on the date of recruitment.

- Outlier diet or anthropometric data (energy intake < 500 or > 5000 kcal/day or BMI < 10 or > 60 kg/m^2)
- Withdrew consent during the study period.
- Biological sex assigned at birth differed to self-reported sex at recruitment.
- Unable to be classified into a diet group (e.g. responded "do not know" or "prefer not to say" for questions on meat or fish intake).

4.0 Exposures

4.1 Primary exposure

The primary exposure of interest will be diet group (regular meat-eater, occasional meat-eater, pescatarian, or vegetarian) – definitions for each diet group are given in Table 1. Due to the small number of vegans in the UK Biobank reported in previous studies (1, 2), vegans will be combined with the vegetarian group for main analyses, and will be considered separately in sensitivity analyses.

Table 1: Diet group categories and definitions.

Diet group	Definition
Regular meat-eater	Total meat intake ≥ 5 servings/week
Occasional meat-eater	Total meat intake < 5 servings /week & > once/month
Pescatarian	Total meat intake ≤ once/month & total fish intake > once/month
Vegetarian	Total meat and fish intakes ≤ once/month, intake of any dairy products or eggs > once/month
Vegan	Total meat, total fish, dairy products, and eggs intake ≤ once/month

4.2 Exposure assessment

Participants will be classified into diet groups based on responses to questions in the touchscreen food frequency questionnaire (FFQ) completed at recruitment that relate to meat, fish, eggs, or dairy consumption. These included questions on frequency of intake of oily fish, non-oily fish, processed meat, poultry, beef, lamb/mutton, pork, eggs, and dairy products. Questions on meat and fish were asked in the form of "how often do you eat [specific food or beverage?]" or similar. Valid options were 1 "never", 2 "less than once a week", "3 once a week", 4 "2-4 times a week", 5 "5-6 times a week", or 6 "once or more daily". We will convert responses to these individual questions into weekly-based consumption frequencies as follows: 0, 0.5, 1, 3, 5.5, 7 servings/week.

We will sum responses to questions on intake of processed meat, poultry, beef, lamb/mutton, and pork to form total meat intake (servings/week); and questions on intake of oily and non-oily fish intake to form total fish intake (servings/week). Intake of eggs and dairy products was assessed by asking

participants “Which of the following do you never eat?”, with options of “Eggs or foods containing eggs”, “Dairy products”, “I eat all of the above”, or “Prefer not to answer”.

Diet groups will be regular meat-eaters (ate meat ≥ 5 times/week), occasional meat-eaters (ate meat < 5 times/week), pescatarians (reported never eating meat but ate fish), vegetarians (reported never eating meat or fish but did consume eggs or dairy products), and vegans (reported never eating meat, fish, eggs, or dairy products).

5.0 Outcomes

The primary outcome will be first incidence of hip fracture (ICD-9 code 820 and ICD-10 codes S72.0-S72.2) identified using hospital inpatient data for England, Scotland, and Wales. This includes Hospital Episode Statistics for England until 30th September 2021, Scottish Morbidity Records for Scotland until 31st July 2021, and the Patient Episode Database for Wales until 28th Feb 2018. The timeframe will be person-years until hip fracture incidence, or until end of study period or death in non-cases, calculated as age at time of event or censoring minus age at study entry. Sources and dates of availability for each region’s hospital inpatient data (and therefore censoring dates) are presented in Table 2.

Table 2: Hospital inpatient data in England, Scotland, and Wales.

Country	**Data source**	**Period of time for which data is available**
England	Hospital Episode Statistics (HES)	1997 – 30th Sept 2021
Scotland	Scottish Morbidity Record (SMR)	1981 – 31st July 2021
Wales	Patient Episode Database for Wales (PEDW)	1998 – 28th Feb 2018

Adapted from: External Info : Data_providers_and_dates (ox.ac.uk)

6.0 Sample size

The minimum detectable hazard ratio for potential associations between each diet group and hip fracture risk (with regular meat-eaters as the reference group) was estimated in Stata (v17.0) assuming the following parameters:

- Total sample size of 472,337 participants, including 247,571 regular meat-eaters, 205,385 occasional meat-eaters, 10,696 pescatarians, and 8685 vegetarians, as has been previously reported in a UK Biobank publication on risk of cancer in these diet groups (1). Numbers of participants in each diet group in our study may differ slightly to the cited UK Biobank study due to differences in exclusion criteria.

- Hip fracture incidence of 3%.
- $p < 0.05$.
- 80% power.
- A theoretical standard deviation of the exposure (diet group) calculated based on the percentage of participants in each diet group (Table 3).

Table 3: Minimal detectable hazard ratios for associations between diet groups and hip fracture risk with regular meat-eaters as the reference group.

Diet group	SD	Minimum detectable HR
Regular meat-eaters	Ref	Ref
Occasional meat-eaters	0.50	1.05
Pescatarians	0.20	1.17
Vegetarians	0.18	1.19
Vegans	0.04	1.82

SD = Standard deviation. HR = Hazard ratio. The SD for dietary group is theoretical and has arbitrary units.

7.0 Descriptive statistics

Dietary, lifestyle, socio-economic, anthropometric, and other relevant characteristics of the cohort at recruitment will be summarised using descriptive statistics (e.g. presenting their means and standard deviations) by diet group, in cases vs non-cases, and in men and women to report any differences in covariates (including co-exposures) between groups at recruitment.

8.0 Statistical modelling

8.1 Main analyses

8.1.1 Absolute risk difference

To estimate the population impact of being in each diet group on hip fracture incidence, we will determine the absolute risk difference for hip fracture between each diet group and regular meat-eaters (reference group) per 1000 people over 10 years. This will be calculated as the crude differences between the predicted incidence per 1000 people over 10 years between each diet group and the regular meat-eaters, using hazard ratios and 95% confidence intervals expressed as floating absolute risks, as described elsewhere (2).

8.1.2 Relative risk

Cox regression models will be applied to estimate HR (95% CI's) for associations between each diet group and hip fracture risk, with regular meat-eaters as the reference group. The proportional hazards assumption will be assessed based on Schoenfeld residuals.

8.1.3 Accounting for confounding

Unadjusted and adjusted cox models will be applied. Both will control for age by using attained age as the timescale. The adjustment set for the adjusted model will be informed by a DAG model, which will be constructed using the online tool DAGitty, following available guidelines on their use (3). Confounders will be considered as covariates that are 1) risk factors of the outcome; 2) associated with the exposure; and 3) not on the causal pathway (4). Potential confounders are age at recruitment, sex, region, ethnicity, socio-economic status or education, marriage, physical activity, smoking, alcohol, BMI (or height and weight), number of children, menopausal status, hormone replacement therapy (HRT) in postmenopausal women, chronic disease prevalence at recruitment, and use of nutritional supplements (either generic or supplement-specific e.g. for calcium, vitamin D, and fish oil in particular, depending on availability of data). A list of likely confounders and their derivation is summarised in Supplementary Table 1.

8.2 Subgroup analyses

We will stratify the adjusted model by potential effect modifiers, and will add these covariates to adjusted models as interaction terms with diet group independently, using likelihood ratio tests comparing regression models with and without interaction terms to test for effect modification.

To investigate the role of diet quality as a potential effect modifier, participants will be dichotomised into higher and lower AHEI adherence groups at the median AHEI score in the overall sample to maximise power in each diet group sub-strata. We will also assess the interaction between diet groups and a five-unit increase in AHEI score on hip fracture risk. Other potential effect modifiers include age (continuous and split at < 60 years, ≥ 60 years), sex (male, female), and BMI (continuous and split at < 18.5, 18.5-24.9, and ≥ 25 kg/m^2 if there is enough power).

The adjusted model will also be further stratified by specific single-nucleotide polymorphisms (SNPs) of genes with a plausible relationship with hip fracture risk and diet (PPARγ, VDR, MTHFR, CYP24A1, CYP27B1, and IGF-1) to explore potential gene-diet interactions.

8.3 Mediation analyses

If a significant association is observed between any diet group and hip fracture in the main analysis (with regular meat-eaters as the reference group), we will further explore the potential mediating

effect of variables that have previously been associated with – or could plausibly be associated with – diet groups and hip fracture risk. Potential mediators include (all measured at recruitment):

- Potential anthropometric mediators: BMI, BMD (at the heel and femoral neck depending on availability of data and number of participants with each measure), bone mineral content, lean muscle mass, hand grip strength, and fat mass;
- Circulating vitamin D and IGF-1 levels;
- Total energy intake and dietary intake of protein, calcium, vitamin B12, and omega-3' fatty acids.

For each potential mediator, we will explore whether it is more appropriate to use measures at recruitment (extrapolated to represent measures during the study period), or to calculate changes in the mediator variable of interest from recruitment to the latest measurement during follow-up, depending on the availability of mediator data in UK biobank participants.

To determine if each of the potential mediators listed vary by diet group, multiple linear regression will be used, adjusting for relevant confounders. We will not explore mediation if there is no significant difference in risk of hip fracture across diet groups, or if the mediator is not significantly different between each diet group and regular meat-eaters.

We will test for mediation using the inverse odds ratio weighting (IORW) method, which estimates the natural direct and indirect effects of potential mediators, and is described in detail elsewhere (5-7).

8.4 Sensitivity analyses

1. An adjusted model with BMI removed from the adjustment set will be presented to determine the contribution of weight management to any associations.

2. We will present an adjusted model with vegans and vegetarians separated to determine if excluding vegans from the vegetarian group alters potential associations with hip fracture risk.

3. Online follow-up 24-hour dietary recall data will be compared with touchscreen FFQ data at recruitment to check for changes in diet group over time. In cases of substantial exposure change over time (e.g., > 10% of participants changing their dietary group), models will be repeated restricted to participants who maintained their original diet group.

4. Excluding cases occurring in the first three years of follow-up to check for reverse causality.

5. Excluding participants on long-term treatment for illnesses at recruitment who may be generally unhealthier and therefore at a higher risk of hip fracture.

6. We will explore patterns of missing covariate data, and will use multiple imputation through Stata's 'mi' package in the event of substantial missing covariate data.

All analyses will be performed using Stata. Two-sided p values < 0.05 will be considered statistically significant. All analyses performed and effect sizes computed will be presented in resulting manuscripts to avoid bias in selection of the reported result.

9.0 Missing data

Participants who are unable to be classified into a diet group will be excluded from the study, such as when participants answered "do not know" or "prefer not to say" to all questions on meat or fish intake, or when participants did not complete these questions. Participants with no hospital record will be excluded. Participants with missing covariate data will be excluded from the main analyses unless the amount of missingness substantially compromises statistical power, in which case unknown categories will be formed for each covariate.

10.0 Timescales

Target analysis completion date (excluding manuscript preparations): Mar 2023.

11.0 Additional information

Fig S1: Equation used to calculate theoretical standard deviations for diet group analyses.

$$\mathrm{SD} = [\mathrm{p}(1 - \mathrm{p})]^{0.5}$$

Where SD = standard deviation and *p* = percentage of participants in the vegetarian category (as a decimal).

11.1 Covariate classification

11.1.1 Socio-demographics

Age at recruitment

Calculated as date of recruitment minus date of birth, truncated to whole year.

Sex

Genetic sex as determined from genotyping analysis.

Region

At recruitment, participants attended one of 22 assessment centres across the UK. We will group the centres into three regions as follows: England (Barts, Birmingham, Bristol, Bury, Croydon, Hounslow, Leeds, Liverpool, Manchester, Middlesborough, Newcastle, Nottingham, Oxford, Reading, Sheffield, Stockport, Stoke), Scotland (Edinburgh, Glasgow), and Wales (Cardiff, Swansea, and Wrexham).

Ethnicity

At recruitment, participants were asked in the touchscreen questionnaire to select their ethnic group among “White”, “Mixed”, “Asian or Asian British”, “Black or black British”, “Chinese”, “Other ethnic group”, “Do not know”, or “Prefer not to say”. We will regroup participants into the following ethnicity categories: White, Mixed race, Asian, Black, Other, and Unknown.

Socio-economic status

Townsend deprivation index will be used to represent socio-economic status. This variable has been created in the UK biobank based on the preceding national census output areas. Each participant was assigned a score corresponding to the output area in which their postcode was located. Participants

will then be split into quintiles from least deprived (Q) to most deprived (Q5), with an additional category for missing data (where postcode information was not provided).

Living alone

In the touchscreen questionnaire at recruitment, participants were asked "Including yourself, how many people are living together in your household?". From this, we will define the variable "live alone" (with options yes, no, unknown).

11.1.2 Anthropometrics

Body mass index (BMI), height, and weight

Body weight and standing height were measured at the assessment centre visit at recruitment. BMI was calculated as a participant's body weight (kg) divided by the square of their height (m).

Other body composition measures

Bioimpedance was measured at the assessment centre visit at recruitment using the Tanita Bc418ma bioimpedance device, from which body fat percentage, whole-body fat mass, whole-body fat-free mass were estimated. Hand grip strength (for each hand) was measured using the Jamar Hydraulic hand dynamometer. Left calcaneal bone mineral density (BMD) was measured using a Norland McCue Contact Ultrasound Bone Analyzer, from which a heel BMD t-score was calculated for each participant. At the second instant of follow-up (2014), a dual energy X-ray absorptiometry (DXA) scan was used to measure left femoral neck BMD (from which femoral neck BMD t-scores were calculated), and total bone mineral content (BMC).

11.1.3 Lifestyle

Physical activity

Physical activity in total metabolic equivalent task (MET) minutes per week was calculated based on a series of questions that asked about frequency and duration of walking, moderate activity, and vigorous activity, and will be used in this study.

Smoking status

At recruitment, participants were asked in the touchscreen questionnaire "Do you smoke tobacco now?" and "In the past, how often have you smoked tobacco?" to determine their smoking status as current, previous, never, or unknown.

Alcohol consumption

In the touchscreen questionnaire at recruitment, participants were asked about their weekly and monthly intake of glasses of red wine, glasses of champagne plus white wine, pints of beer plus cider, measures of spirits or liqueurs, glasses of fortified wine, and glasses of other alcohol. We will sum participants weekly and monthly alcohol intakes, respectively. Weekly-based total alcohol intakes will be converted into daily total alcohol intake (drinks/day). For those with missing weekly-based alcohol intakes for any specific type, monthly-based intakes will be used.

Participants with missing data for weekly and monthly intake of specific alcohol types will be considered non-consumers. Non-consumers of total alcohol intake (drinks/day) will be cross-checked with responses to a question in the touchscreen questionnaire at recruitment that asked "how often do you drink alcohol?" with options of 1 "daily or almost daily", 2 "three or four times a week", 3 "once or twice a week", 4 "one to three times a month", 5 "special occasions only", 6 "never", or 7 "prefer not to answer".

Diet and nutrition

Alternative Healthy Eating Index Score, energy intake, and nutrient intakes

The Alternative Healthy Eating Index (AHEI) will be used as an index of diet quality. Adherence to the AHEI has previously been inversely associated with risk of hip fracture, and could plausibly vary by diet group (8).

The AHEI awards between 0-10 points for 11 food and nutrient components. Points are awarded for 1) higher intakes of vegetables, whole fruits, nuts and legumes, wholegrains, polyunsaturated fatty acids, and n-3 fatty acids; 2) lower intakes of red and processed meats, sugar-sweetened beverages and fruit juices, trans fats, and sodium; and 3) moderate intake of alcohol. The total possible score ranges from 0-110 points. Table S1 shows how each food or nutrient component will be calculated and scored (9).

Component	**Criteria for minimum score (0)**	**Criteria for maximum score (0)**	**Component derivation**
Vegetables, servings/day	0	≥ 5	Participants were asked in the touchscreen questionnaire at recruitment how many heaped tablespoons of cooked vegetables and raw vegetables/salad they ate per day, respectively. These will be summed to give total vegetable intake, and two heaped

			tablespoons will be considered as one serving of vegetable intake.
Fruit, servings/day	0	≥ 4	Participants were asked in the touchscreen questionnaire at recruitment how many pieces of fresh fruit and dried fruit they ate per day, respectively. These will be summed to give total fruit intake. One piece of fresh fruit and two pieces of dried fruit will each be considered a serving of fruit, respectively.
Wholegrains, g/day			Intake of wholegrains (g/day) has been previously calculated using 24-hour dietary recall data, and will be used in this study.
Women		75	
Men		90	
Sugar-sweetened beverages and fruit juice, servings/day	≥ 1	0	Intake of sugar-sweetened beverages and fruit juice (both in g/day) have been previously calculated using 24-hour dietary recall data. Each will be divided by standard portion sizes, then will be summed to give total intake of sugar=sweetened beverages and fruit juice (servings/day)
Nuts and legumes, servings/day	0	≥ 1	Intakes of legumes and pulses, salted nuts and seeds, and unsalted nuts and seeds (each in g/day) have been previously calculated using 24-hour dietary recall data. Each will be divided by standard portion sizes, then will be summed to give nuts and legumes intake (servings/day).
Red/processed meat, servings/day	≥ 1.5	0	Participants were asked in the touchscreen questionnaire at recruitment how often they ate processed meat, beef, lamb/mutton, and pork, respectively. Responses to these questions will be converted to daily-based consumption frequencies, and will be summed

			to give total red and processed meat intake in servings per day
Trans fat, % of energy	≥ 4	≤ 0.5	Trans fat intake (g/day) and total energy intake (kJ/day) have been previously estimated using 24-hour dietary recall data. We will calculate trans fat (% energy) using these two s, and conversion rates of 1 kJ = 4.184 kcal and 1 g trans fat = 9 kcal, respectively.
omega-3 fats (EPA +DHA), mg/day	0	250	Omega-3 fatty acid intake (g/day) has been previously estimated using 24-hour dietary recall data.
PUFA, % of energy	≤ 2	≥ 10	PUFA intake (g/day) and total energy intake (kJ/day) have been previously estimated from 24-hour dietary recall data. We will calculate PUFA (% energy) using these two s, and conversion rates of 1 kJ = 4.184 kcal and 1 g PUFA = 9 kcal, respectively.
Sodium, mg/day	Highest decile	Lowest decile	Previously estimated using 24-hour dietary recall data (mg/day).
Alcohol (drinks/day)			
Women	≥ 2.5	0.5 – 1.5	
Men	≥ 3.5	0.5 – 2.0	
Total	0	110	Sum each individual component score per participant.

Scoring based on available guidelines for calculating Alternative Healthy Eating Index Score (9), and dietary nutrient intakes based on previous calculations from 24-hour dietary recall data (10).

Where possible, we will use dietary data collected from the touchscreen questionnaire to estimate food intakes. Total energy intake and dietary nutrient intakes, including protein, calcium, and omega-3 fatty acid intakes, were previously calculated from 24-hour dietary recall data, and will be used in this study (10).

Circulating vitamin D and insulin-like growth factor 1 (IGF-1) levels

Circulating IGF-1 and vitamin D levels (both in nmol/L) were measured by CLIA analysis on a DiaSorin Ltd LIASON XL.

Use of nutritional supplements

At recruitment, participants were asked in the touchscreen questionnaire if they regularly take any of the following supplements: vitamin A, vitamin B, vitamin C, vitamin D, vitamin E, folic acid or folate (vitamin B9), multivitamins or minerals, none of the above, or prefer not to answer. From this, we will define the variable "use of nutritional supplements" with options of yes, no, or unknown.

In the online 24-hour recall assessment of diet, participants were also asked "Did you have any vitamin or mineral supplements yesterday", with more detailed options available. We will regroup responses to this question into the following supplementation categories: "Calcium", "Vitamin D", "Fish oil", "Vitamin B12", "Multivitamin", "Other", or "None", or "Unknown".

11.1.4 Other

Number of children

At recruitment in the touchscreen questionnaire, women were asked "how many children have you given birth to?". This will be treated as a continuous variable. This variable is not applicable to men.

Menopausal status

At recruitment in the touchscreen questionnaire, women were asked multiple questions relating to menopausal status. Women will be defined as premenopausal or postmenopausal at recruitment using the following criteria:

Premenopausal: answered "no" to the question that asked about having gone through menopause, or answered "not sure", and:

- Were < 55 years old, did not report having a bilateral oophorectomy or hysterectomy, and did not report using hormone replacement therapy (HRT), or
- Were < 55 years old, did not report having a bilateral oophorectomy or hysterectomy, and reported menstruating on the day of recruitment

Postmenopausal: answered "yes" to having gone through menopause, or answered "not sure" and were ≥ 55 years old or had a bilateral oophorectomy.

Menopausal hormone replacement therapy (HRT)

At recruitment in the touchscreen questionnaire, women were asked "Have you ever used hormone replacement therapy?" and if yes, "How old were you when you last used hormone replacement therapy?". We will categorise HRT use based on these questions as "Current", "Former", "Never", or "Unknown".

Chronic disease prevalence at recruitment

We will identify prevalence of chronic diseases that could act as confounders using both self-reported information from questions on health and medical history asked in the touchscreen questionnaire, and through use of hospital records (with the date of diagnosis being before or on the date of recruitment). Relevant chronic diseases that will be considered confounders will be informed by existing literature and by use of a Directed Acyclic Graph (DAG), but will likely include hip fracture, other fracture, osteoporosis, diabetes, cardiovascular disease, and cancer.

12.0 References

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