Long-Term Intake of Animal Flesh, Fruits and Vegetables and the Incidence of Hypertension in Three Prospective Cohort Studies

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Abstract:

Vegetarian and vegan diets are associated with a lower prevalence of hypertension when compared with animal-based diets. Small, short-term interventional studies showed that replacing an omnivorous diet with a vegetarian diet lowered blood pressure. The potential mechanisms by which plant-based food intake is linked to a reduced risk of hypertension are not well understood. However, proposed effects include systemic inflammation, endothelial dysfunction, and the activation of the renin-angiotensin system (RAS) among those eating animal-based foods. We propose the VEGIE-BP (VEGetarian Initiative on the Endpoint of Blood Pressure) study, a prospective longitudinal study examining the incidence of hypertension in three large-scale, ongoing prospective cohorts with decades of follow-up: the Nurses' Health Study 1 (NHS1), the Nurses' Health Study 2 (NHS2), and the Health Professionals Follow-up Study (HPFS). These cohorts have validated information about health outcomes, as well as longitudinal and repeated measures of diet using detailed food-frequency questionnaires. Biomarkers of inflammation and endothelial function have also been measured on many of these participants, and at several time points in some participants. These cohorts therefore represent a unique resource to analyze the longitudinal associations of vegetarian/vegan diets and specific foods with the incidence of hypertension as well as associations with inflammation and endothelial dysfunction. In addition, we will explore the possible mechanisms of vegetarian diets, using not only these pre-existing inflammatory and endothelial biomarkers from the three cohorts, but also by examining the associations of diet with gold-standard measurements of endothelial function and RAS activation in 225 individuals enrolled the Modifiable Effectors of Renin System Activation Treatment Evaluation (MODERATE) trial. VEGIE-BP is the first prospective, longitudinal study examining the relation of vegetarian/vegan diets and specific foods on the incidence of hypertension and its potential mechanisms. From a public health perspective, this study will advance the understanding of how vegetarian diets are related to blood pressure and will help patients to avoid hypertension, chronic kidney disease and cardiovascular disease.

1. **Specific Aims:**

Two small, short-term feeding trials and two large cross-sectional studies found that adults consuming a vegetarian diet had lower blood pressure than those consuming an omnivorous diet. However, there are no large-scale prospective studies assessing whether a long-term plant-based diet is associated with a reduced risk of developing hypertension. In addition, few studies have addressed possible mechanisms that could underlie this potential relation. We hypothesize that long-term vegetarian/vegan diets prevent the development of hypertension and have less systemic inflammation, endothelial dysfunction, and activation of the RAS (all mechanisms of hypertension). We propose the VEGIE-BP (VEGetarian Initiative on the Endpoint of Blood Pressure) study, a prospective longitudinal study examining the incidence of hypertension in three large-scale, ongoing prospective cohorts with decades of follow-up and detailed food-frequency questionnaires and health outcomes: the Nurses' Health Study 1 (NHS1), the Nurses' Health Study 2 (NHS2), and the Health Professionals Follow-up Study (HPFS). In addition, information on diet, gold-standard assessments of endothelial function and RAS activation are available in the Modifiable Effectors of Renin System Activation Treatment Evaluation (MODERATE) trial (HL105440). These hypotheses give rise to the following aims:

1. **Vegetarian/vegan diets and incidence of hypertension**

a. Among individuals from NHS1 (N=121,700), NHS2 (N=116,430) and HPFS (N=51,529), we will examine the prospective association of vegetarian diets with the risk of hypertension.

b. We will also explore how specific plant-based foods (e.g., nuts, berries, and green leafy vegetables) and specific animal-based foods (e.g., red and processed meat, poultry, and fish) are associated with the incidence of hypertension.

2. **Vegetarian/vegan diets and mechanisms of hypertension**

a. In participants with pre-existing biomarker measurements in the three cohorts, we will analyze the relations of vegetarian/vegan diets with inflammatory and endothelial biomarkers, specifically c-reactive protein (N=20,148), interleukin-6 (N=14,251), tumor necrosis factor-α receptor 2 (N=6,159), E-selectin (n=6,159), I-CAM 1 (n=8,396), and V-CAM 1 (n=3,949).

b. We will examine associations of short-term and long-term diets with gold-standard measurements of endothelial function and RAS activation in the MODERATE trial.

2. a. Background:

A.1.a.Vegetarian/Vegan diets and Hypertension**:** two large cross-sectional studies showed that vegetarian diets are associated with a lower prevalence of hypertension[1,2]. In the European Prospective Investigation into Cancer and Nutrition (EPIC–Oxford) cohort study, for example, the age-adjusted self-reported hypertension prevalence ranged from 15% in male meat eaters to 5.8% in male vegans[1]. In addition, a small, 6-week study found that systolic and diastolic pressures fell significantly when participants switched from an omnivorous diet to a vegetarian diet [3,4]. However, we know of no prospective, longitudinal studies examining whether long-term vegetarian/vegan diets are associated with a reduced incidence of hypertension.

A.1.b Specific foods and Hypertension: the association of broad plant-based food categories such as fruits, vegetables, whole grains, or others with the development of hypertension was examined in three prospective cohorts and, in general, the findings were consistent with an inverse relationship of fruits, nuts, and whole grains with hypertension[5-7]. It may also be important to examine more categories of plant-based foods. As an example, when we investigated the association of flavonoids with hypertension in participants from NHS1, NHS2, and HPFS, a secondary analysis suggested that a high intake of anthocyanins (but not other flavonoids) was related to an 8% reduced risk[8]. Since blueberries and strawberries contain the most anthocyanins, this suggests that certain fruits (as opposed to all fruits) may offer the greatest benefit in terms of blood pressure. Also, in the NHS1 and HPFS, eating nuts five or six times per week was inversely associated with cardiovascular and total mortality (RR, 0.85; 95% CI, 0.79 to 0.91 for total mortality)[9]. An inverse association of vegetarian/vegan diets with hypertension might also result from avoidance of animal products. Red meat, for example, was found to be related to a higher incidence of hypertension[10-12]. The associations of other animal products, such as fish and poultry, with hypertension risk remain controversial[10,13,14].

A.2. Vegetarian/vegan diets and mechanisms of hypertension: few studies have addressed the potential mechanisms that could underlie a relationship between vegetarian diets and low blood pressure, although activation of the renin-angiotensin system (RAS) and improved endothelial function have been proposed[15]. One study focused on the vasodilatory effects of plant-based diets, showing an increased flow-mediated and nitroglycerin-induced

vasodilatation of brachial arteries in vegetarians as compared with omnivores[15,16]. Vegetarians may also have lower circulating levels of endothelial dysfunction biomarkers, such as E-selectin and cICAM-1[17]. In a cross-sectional population based study, vegetarians had lower circulating levels of C-reactive protein (CRP)[18]. This is of clinical importance because inflammation, marked by elevated levels of markers such as CRP, has been proposed as a potential mechanism for hypertension as well as for cardiovascular events[19,20].

2 .b. Significance:

Although cross-sectional data exist to support the hypothesis that vegetarian diets are associated with a lower prevalence of hypertension, there are no longitudinal studies examining the incidence of hypertension with vegetarian diets. Also, data are limited analyzing long-term consumption of specific plant and animal based foods and hypertension risk, and examining potential hypertension mechanisms associated with vegetarian/vegan diets. The goal of this project parallels the mission of the American Heart Association; indeed, given the tremendous public health importance of hypertension, one of the risk factors of cardiovascular disease and stroke, VEGIE-BP will aim to advance the understanding of how vegetarian diets are related to blood pressure and will help patients achieve healthier lives.

3. Preliminary Studies:

In order to document the feasibility of our specific aims, we performed preliminary analyses of one of the three large cohorts (NHS2, N=116,430) to explore the association of different animal products with the incidence of hypertension. In this cohort, 22,157 of 97,991 initially non-hypertensive women developed hypertension during 18 years of follow-up. Eating more than one serving of red meat per day (compared with <once/month) was significantly associated with a higher risk of developing hypertension (see Table) after adjusting for age, BMI, physical activity, family history of hypertension, alcohol, sugar-sweetened beverage intake, artificially-sweetened beverage intake, and mutually for each animal-based product listed. Interestingly, poultry did not show any association with the risk of developing

hypertension. Seafood on the other hand, including shellfish and fish, was associated with an increased risk of hypertension after 18 years of follow up, in contrast to other smaller studies with fewer assessments of dietary intake. Further analyses will help describe these relationships in more details. In addition, we performed a preliminary analysis of vegan diets in this cohort; compared with non-vegetarians, vegans had a substantially reduced risk of developing hypertension (0.38; 95% CI, 0.14-0.98).

Animal Product (frequency)	Hazard Ratios and 95% CI
Poultry (>1/day)	0.93 (0.84-1.04)
Red meat (>1/day)	1.28 (1.17-1.40)
Seafood (>1/day)	1.14 (1.01-1.28)
All processed meat (3x/week to 1/day)	1.09 (1.04-1.15)

In a preliminary analysis of the first 57 participants in MODERATE with complete data on their first baseline visit, those who did not eat animal products in the day prior to the study had significantly better endothelial function than those who did eat meat (β=2.99%; p=0.04), after controlling for age, BMI, and systolic BP.

4. Research Design and Methods:

A. Study Populations and Study Design

Specific aim 1 will be addressed using a prospective cohort study design. The source populations include the NHS1 (N=121,700, aged 30-55 in 1976), NHS2 (N=116,430, aged 25-42 in 1989), and the HPFS (N =51,529, aged 40-75 in 1986). Follow-up in these cohorts is extensive (37, 24 and 27 years, respectively) and loss to follow-up is less than 10%. Participants return a detailed questionnaire every two years documenting numerous health-related factors and medical events (including a clinician-diagnosis of hypertension). Semi-quantitative food frequency questionnaires (FFQs) are answered every four years; previous work has shown these FFQs to be both reproducible and valid [21,22].

All participants in the NHS 1 and NHS 2 cohorts are women and all participants in the HPFS cohort are men; the three cohorts have no restrictions on race and socioeconomic status.

Specific aim 2 will be cross-sectional and make use of NHS1, NHS2, and HPFS to examine biomarkers of inflammation and endothelial function. Blood samples were collected, frozen, and stored from large subsets of participants from these cohorts: 32,826 participants of NHS1 in 1989; 29,616 participants of NHS2 in 1997; and 18,025 participants of HPFS in 1993. From these participants with frozen biospecimens, inflammatory and endothelial biomarkers have *already been measured* on a large number of individuals, specifically: CRP (N=20,148); interleukin-6 (IL6, N=14,251); tumor necrosis factor-α receptor 2 (TNFR2, N=6,159), E-selectin (N= 6,159), I-CAM 1 (N= 8,396), and V-CAM 1 (N= 3,949).

In addition, for specific aim 2 the source population will consist of overweight and obese participants enrolled into the MODERATE trial. MODERATE (final N=225) is an NIH-funded randomized trial examining the effects of vitamin D supplementation and uric acid lowering on various physiologic endpoints, including endothelial function, 24-hour ambulatory BP parameters, and the RAS; biological specimens, including urine, are also collected. Individuals who are overweight and obese (two-thirds of US adults) represent an important population who are known to have lower 25(OH)D levels, higher uric acid concentrations,

activation of the RAS, endothelial dysfunction, and an increased risk of hypertension, cardiovascular disease and chronic kidney disease.

In the MODERATE study, men and women are included, as well as all races and ethnicities. Women who are pregnant are excluded as certain medications used for the physiological interventions are contraindicated during pregnancy.

B. Exposures

Using information from the FFQs discussed above, the primary exposure for specific aim 1 will be a dietary food index to categorize vegetarians/vegans and omnivores. This index will be modeled after the Adventist Vegetarian Index described in a recent publication[23]. This index has five categories: vegans (who consume eggs, dairy, fish, poultry, and all other meats less than once/month); lacto-ovo-vegetarians (who consume eggs and dairy once/month or more, but fish, poultry, and other meats less than once/month); pesco-vegetarians (who consume fish once/month or more, but poultry and other meats less than once/month); semi-vegetarians (who consume poultry and other meats once/month or more but who consume fish, poultry, and other meats less than once/week), and non-vegetarians (who consume fish, poultry, and other meats at least once/week). This index will be used in collaboration with Dr. Frank Hu at the Harvard School of Public Health. Dr. Hu and his team are currently working on developing the index.

Other exposures for specific aim 1 will include the frequency that broad plant-based food categories are consumed, specifically fruits, vegetables, legumes, whole grains, and refined grains, as well as the frequency that broad animal-based food categories are consumed, specifically dairy products, eggs, and animal flesh. We will also examine substantially more granular categories of these broad groups. As examples, we will compare berries with other types of fruits, compare nuts with other legumes, compare green leafy vegetables with other vegetables, and compare red and processed meat with fish, poultry, dairy, and eggs. These exposures will be initially categorized into quintiles of intake, and then into more practical groupings.

The exposures for specific aim 2 will differ depending upon the whether we are examining circulating inflammatory and endothelial biomarkers (NHS1, NHS2, and HPFS) or gold-standard measures of RAS activation and endothelial function (MODERATE). For NHS1, NHS2, and HPFS, we will employ the same dietary variables as for specific aim 1. For MODERATE, we will have two exposures: long-term dietary pattern (3 categories: omnivore, vegetarian, vegan), which is self-reported by participants, and short-term diet (ascertained by 24-hour recall [number of servings/day] and by urinary biomarkers [which can be modeled as continuous variables]). These urinary biomarkers, specifically urinary taurine, and both 1- and 3-methylhistidine, are validated markers of animal product intake[24,25] and will be measured in all MODERATE participants during the course of grant period (paid for by the parent MODERATE fund).

C. Covariates

We will determine whether or not associations are independent by carefully controlling for potential confounding factors. In all four cohorts (NHS1, NHS2, HPFS, MODERATE), the following important variables are ascertained and will be included in our models: age, BMI, physical activity, alcohol intake, cigarette smoking, use of certain medications (eg, hormone use among women, cholesterol medications) and other dietary factors including sugar-sweetened beverage intake, sodium and potassium intake, and total calorie intake. In NHS1, NHS2, and HPFS, such information is collected every two years during follow-up. In MODERATE, this information is collected either within the month prior to or at the same time as the inpatient physiologic evaluation; additionally, 24-hour ambulatory blood pressure measurements are available in MODERATE and can be used in our models.

D. Outcomes

The primary outcome for specific aim 1 will be self-reported incident hypertension. In all three large cohorts, participants are asked on the biennial questionnaires to report whether they have received a diagnosis of hypertension from their clinician during the preceding two years, and to also report when that diagnosis was made. Self-reported hypertension by these health professionals has been shown to be valid[26-28]. In 51 cases of self-reported hypertension in

NHS1, for example, 77% had a pressure >160/95 and 100% had a pressure >140/90[26]. In NHS2 and HPFS, the medical record review of randomly chosen participants confirmed the diagnosis of hypertension in 94% and 100%, respectively[27,28].

The outcomes for specific aim 2 include pre-existing biomarkers from NHS1, NHS2, and HPFS (CRP, IL6, TNFR2, E-selectin, ICAM-1, VCAM-1), and gold-standard measures of endothelial function and RAS activation in MODERATE. In MODERATE, endothelial dependent and independent vasodilation are ascertained with brachial ultrasound[29,30]. Activation of the RAS is ascertained in all participants while in controlled high-sodium balance using measures of plasma renin activity and angiotensin II (to assess the circulating RAS), and the renal plasma flow response to captopril (to assess the intrarenal RAS).

E. Statistical Analysis

For specific aim 1, we will exclude individuals who have prevalent hypertension at the time of the first dietary questionnaire, such that our analyses will be prospective. We will than group individuals into the five categories described above (vegan, lacto-ovo-vegetarian, pesco-vegetarian, semi-vegetarian, non-vegetarian). The reference group will be non-vegetarians. Independent prospective associations between these diets and incident hypertension will be analyzed with multivariable Cox proportional hazards regression (adjusted for the covariates mentioned above in C). A similar approach will be used to analyze broach and granular plant and animal-based foods. In these Cox models, person-time counted from time date the FFQ was returned by the participant to the date of hypertension diagnosis, the date of death, or the end of follow-up, whichever occurs first, and allocated according to exposure status. The hazard ratios from the three cohorts will be pooled using random-effects meta-analysis.

An enormous advantage of the NHS 1 and 2 and the HPFS cohorts is the fact that *diet is ascertained multiple times* during the course of follow-up. The updated dietary information that participants provide by non-censored individuals will be incorporated into our analyses in two ways: updating with replacement (in which exposure status is changed to reflect the most up-to-date data); and cumulative averaging (in which exposure status is a weighted average of all prior and current data).

For specific aim 2, we will test the inflammatory and endothelial biomarkers from NHS1, NHS2, and HPFS for normality, and log-transform those that are not normally distributed. We will then employ multivariable linear regression, with biomarkers as the dependent variable and dietary exposures (as in aim 1) as the independent variables. In MODERATE, endothelial function and the measures of RAS activation will be the dependent variables (log-transformed if needed), and long-term and short-term dietary measures (described above in B) will be the independent variables. Covariates can include those discussed above in C.

F. Power and Sample Size

NHS2 has the smallest number of cases among the three cohorts (22,157 cases; the other cohorts have more), and is therefore the least well powered of the three. Thus, we estimated power for NHS2, and our power will be greater for the other two cohorts, and of course will be greatest when these three results are pooled using meta-analysis. Although slightly different from one questionnaire cycle to the next, approximately 1.1% of participants in NHS2 were lacto-ovo-vegetarians, and approximately 0.2% of participants were vegan. Given this information and a 2-sided p-value of 0.05, we will have 80% power to detect a hazard ratio of 0.86 among lacto-ovo-vegetarians and a hazard ratio of 0.65 among vegans. Based upon our preliminary results (see above), we will have sufficient power to detect reasonable associations. For the pre-existing biomarkers in specific aim 2, we computed power for the biomarker with the smallest sample size and from only one of the three cohorts (ie, ICAM-1 in NHS2). This is a very conservative power estimate since the actual sample size will be larger when all three cohorts are pooled and for the other biomarkers. In NHS2, 1,923 participants have pre-existing ICAM-1. With a 2-sided p-value of 0.05 and if 1.1% are lacto-ovo-vegetarians, then we will have 80% power to detect difference in ICAM-1 as small as 2.88 ng/mL difference in ICAM 1 concentration among lacto-ovo-vegetarians compared with non-vegetarians. To put this in perspective, pharmaceuticals that are known to improve endothelial function, such as the angiotensin-converting enzyme inhibitors, lower ICAM-1 levels to a larger degree (by 16 ng/mL in one study)[31]. Thus, we will have more than enough power to detect clinically important differences in biomarker levels.

Of the first 161 participants enrolled in MODERATE who had completed their baseline visit, 12.9% reported no intake of meat, poultry, seafood, or eggs in the preceding 24 hours. If future participants are similar, then in the final population of 225 individuals, 27 will not have consumed animal products in the day prior to their study. Using these numbers, as well as the standard deviation of endothelial function (ie, endothelial dependent vasodilation as a % of baseline) among the subset who have completed their baseline visit (which is 4.4%), and a 2-sided p-value of 0.05, we will have at least 80% power to detect a difference in endothelial function of 2.4%. This improvement is similar to exercise-induced improvements in endothelial function[32].

5. Ethical aspects of the proposed research:

The pre-existing data from NHS1, NHS2, and HPFS include biomarker data and validated, detailed food-frequency questionnaires and health outcome information. As for the MODERATE data, the only individually identifiable private data that will be collected are names, addresses, and contact phone numbers so that participants can be screened with follow-up phone calls. This personal information, however, will not be associated with the results obtained from the various tests performed, as the latter will be associated with a study ID number. The file linking the study ID number and the personally identifiable information will be kept by the PI in a secure location and will only be violated if medically necessary.

The potential risks to participants in the MODERATE trial include: (i)potential side effects from being on a high salt diet for 3 days, such as elevation of blood pressure or hypokalemia; (ii)potential side effects from medications administered during the inpatient stays, including dizziness, nausea, or hypotension from sublingual nitroglycerine (used for the endothelial function study), or from captopril administration (for assessing RAS activity); (iii)side effects from study medications. To protect against potential risks, individuals at increased risk for adverse events from the study medications will be excluded from enrollment into the study. Weekly phone calls will be made to assess for adverse events, and biochemical side effects will be monitored through laboratory testing. Risk associated with inpatient procedures will be minimized by continuous monitoring by a physician and a nurse.

As further described in the MODERATE trial grant application, the process of informed consent will provide potential participants with the following: purpose of the study, drugs to be administered and the possible use of placebo, a description of randomization and blinding, reasons why the participant may not be eligible for the study, the number of participants expected to be enrolled, the funding source and duration of the study, a detailed description of all study protocols and tests to be performed, storage of blood samples for possible future use, circumstances under which participation may be terminated by the investigator, procedures for safe and orderly termination if the participant wishes to withdraw, a detailed explanation of all potential risks, including unforeseen risks, potential benefits and remuneration.

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