1. Full Title: Impact of Dietary Intake of Vitamins A and D on Blood Pressure and Hypertension: The ARIC Study

b. Abbreviated Title (Length 26 characters): Vitamin A, D and Hypertension

2. Writing Group:

Writing group members: Anthony R. Mawson, Alan Penman, Kenneth Butler, M. Iftekhar Ullah, Tom Mosley, Jr. (others welcome)

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _TM_ [please confirm with your initials electronically or in writing]

First author: Anthony R. Mawson
Address: Division of Genetics
Department of Pediatrics
University of Mississippi Medical Center
2500 N. State Street, Room N-527
Jackson, Mississippi 39216

Phone: 601 984 1927    Fax: 601 984 1924
E-mail: amawson@prevmed.umsmed.edu

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).

Name: Alan Penman
Address: Division of Geriatrics, Department of Medicine
University of Mississippi Medical Center
2500 N. State Street
Jackson, Mississippi 39216

Phone: 601-815-5836    Fax: 601-815-3422
E-mail: apenman@medicine.umsmed.edu

3. Timeline 12-18 months.
4. Main Hypothesis/Study Questions:
The role of individual nutrients and their interactions in blood pressure regulation and the
development of cardiovascular disorders (CVD) is not well understood. The extent to which food
and supplement intake can explain ethnic differences in blood pressure is also uncertain. Vitamin
A (retinol) in particular has been little explored in relation to cardiovascular disease and
associated risk factors. Vitamin A (vA) is considered an antioxidant vitamin and is essential in
low concentration for many biological functions including embryonic development of the heart
and vasculature (Pan and Baker, 2007), but in higher concentration vitamin A can be prooxidant,
cytotoxic, mutagenic and teratogenic (Jetten, 1983; Livrea 1996; Penniston and Tanumihardjo,
2006). Some studies suggest that increased blood concentrations of retinoids (vA and its
congeners) as well as vA supplements, are associated with hypertension and CVD. However, the
contribution of vitamin A-containing foods and supplements to these health outcomes is
uncertain. vA also affects and is affected by other dietary factors, in particular vitamin D (vD),
low blood levels of which are associated with hypertension (Lee 2008; Ullah et al., 2009). vA
and vD are inversely related and mutually inhibitory (Johansson and Melhus, 2001). The effects
of each vitamin thus depend partly on the intake or availability of the other. Since most vD is
generated in the body by exposure to sunlight and only a little is obtained from the diet, chiefly
from fortified foods, the association between vD deficiency and hypertension could be partly due
to amplification of the effects of vA or to inhibitory effects on vD resulting from high intakes or
concentrations of vA. On the other hand, high dietary intakes or serum concentrations of vD
could offset high vA intakes or concentrations and be associated with low or normal blood
pressure.

Study Objective:
To determine the separate and combined effects of vA and vD dietary intake on blood pressure
and hypertension in the biracial Atherosclerosis Risk in Communities (ARIC) study population.

Specific Aims:
1. To test the hypotheses that:
   1.1. vD dietary and/or supplement intake is inversely associated with BP and hypertension,
       whereas
   1.2. vA dietary/supplement intake is positively associated with BP and hypertension;
   1.3. the combination of low vD intake (lowest quintile) plus high vA intake (highest quintile) is
       associated with increased blood pressure and a greater risk of hypertension than either low
       vD or high vA intake alone; and, in view of mutually inhibitory effect of vA and D:
       1.4. the combination of high vA and high vD intakes are associated with normal BP.

2. To determine the extent to which the combined impact of a high dietary intake of vA and a
   low intake of vD accounts for hypertension after controlling for caloric intake.

5. Rationale:
Vitamin A
Vitamin A (vA) is supplied by the diet either from carotenoid-containing plants and vegetables,
which are converted to retinol (vA alcohol), or preformed from liver, fish, eggs, and dairy
products. Retinol is stored primarily (80-90%) in the liver and transported to the target tissues by
retinol-binding protein (RBP), where it is converted from retinol to retinoic acid (RA), the active
form of vitamin A in most cellular differentiation systems. RA serves as a ligand for specific retinoid receptors (retinoic acid receptors [RARs] and retinoid X receptors [RXRs]) that regulate the transcription of many target genes (Blomhoff and Blomhoff, 2006; Litwak, 2007). Vitamin A toxicity can occur due to excessive dietary consumption or from treatment with retinoids. Retinoic acid and other acidic retinoids are more biologically active and hence toxic than retinol, but the precise ranges of serum retinoic acid associated with symptomatic acute or chronic vitamin A toxicity are not well defined (Penniston and Tanumihardjo, 2006).

The question of whether high dietary intakes of vA (or vA toxicity) are associated with hypertension and CVD is presently uncertain. Although vA supplementation has been found in numerous studies to reduce childhood mortality in undernourished populations (Semba, 1999), the reported effects of long-term supplementation on cardiovascular and other health risks in well-nourished adults are contradictory (Duvall, 2005; Riccioni et al., 2007). The Nurses’ Health Study, a prospective study involving 34,486 women that used questionnaires to evaluate the health impact of adding vA to regular diets, found no association between dietary supplements of vA and the risk of coronary disease (Kushi et al., 1996). In the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, over 29,000 male smokers were randomized to 50 IU/d of vitamin E, 20 mg/d of β-carotene, both, or placebo, and followed for 5–8 years for the development of lung cancer and major coronary events. Mortality from both conditions was increased (ATBC Cancer Prevention Study Group, 1994). The Beta-Carotene and Retinol Efficacy Trial (CARET) tested the effects of combined treatment with β-carotene (30 mg/d) and vA (retinyl palmitate) in 18,000 patients with a history of cigarette smoking or exposure to asbestos (Omenn et al., 1996). This study also found increased risks of lung cancer and ischemic heart disease. The Physicians’ Health Study enrolled 22,000 US male physicians and randomized them to β-carotene (50 mg every other day), aspirin (325 mg/d), both, or neither, and followed them for 12 years. No effect was seen with β-carotene on the risk of MI, stroke, or cancer (Hennekens et al., 1996). The use of topical tretinoin, a commonly used retinoid cream, was found to be associated with an increased risk of all-cause mortality, mainly from vascular disorders, in the Veterans Affairs Topical Tretinoin Chemoprevention Trial. However, the authors expressed doubt that the association was causal (Weinstock et al., 2009).

With regard to dietary intake of vA-containing foods, present evidence suggests that high intakes are associated with a reduced risk of hypertension and CVD (Lane et al., 2008; Zulet et al., 2008; Bhat and Manolescu, 2008). On the other hand, increased serum vA concentrations appear to be associated with hypertension. For example, Chen et al. (2002) examined the association between serum vA, β-carotene levels and hypertension among 15,317 men and women ≥20 years of age who participated in the Third National Health and Nutrition Examination Survey (NHANES III). In multivariate models, a 1 SD difference in vA (16.2 µg/dL) was associated with a 43% increased odds of hypertension (OR, 1.43; 95% CI, 1.34 to 1.53). Serum vA was positively and significantly associated with both systolic and diastolic blood pressure, whereas β-carotene was inversely and significantly associated with systolic but not diastolic blood pressure.

Hypertension is a feature of the metabolic syndrome that includes insulin resistance, obesity, hypertriglyceridemia and hyperuricemia. Serum levels of RBP4, a protein secreted by adipocytes, are increased in insulin-resistant states in mice (Yang et al., 2005) and correlate with the magnitude of insulin resistance in human subjects with obesity, impaired glucose tolerance,
or type 2 diabetes. Elevated serum RBP4 is also associated with other components of the metabolic syndrome, including increased systolic blood pressure, body-mass index, waist-to-hip ratio, serum triglyceride levels, and decreased high-density lipoprotein cholesterol levels (Graham et al., 2006). In a study of 885 patients with type 2 diabetes mellitus (Chen et al., 2009), serum RBP4 correlated positively with systolic blood pressure, age, waist circumference, waist-to-hip ratio, total cholesterol, triglyceride, uric acid, creatinine, and urine albumin-to-creatinine ratio. RBP4 level was independently associated with raised uric acid level, a well established risk factor for hypertension and hypertension-associated morbidity (Feig et al., 2008).

Hypertriglyceridemia is also a risk factor for hypertension and, like insulin resistance, can be induced by synthetic vA compounds (e.g., isotretinoin, 13-cis-RA) and high doses of vA (Sedova et al., 2004). Patients with hypertension exhale significantly less nitric oxide (NO) than healthy controls (Schilling et al., 1994). NO and retinoic acid are inversely related (Mehta et al., 1994; Sirsjo et al., 2000), e.g., retinoids inhibit NO synthesis in vascular smooth muscle (Hirokawa et al., 1994). Reports of low levels of NO in hypertension are consistent with the hypothesis that retinoids contribute to the disorder by impairing vasodilation.

**Vitamin D**

Fat-soluble vD is present in a very few foods but is produced endogenously by ultraviolet (UV-B) radiation from sunlight, which triggers vD synthesis. The best food sources for vD are salmon, tuna and mackerel and fish liver oils; small amounts are found in beef liver, cheese and egg yolks. Fortified foods provide most of the vD in the American diet (Institute of Medicine, 1997). vD nutritional needs are mostly met by exposure to sunlight via UV-B radiation (Cranney et al., 2007).

Evidence is growing that vD plays a role in hypertension and the regulation of blood pressure (Krause et al., 1998; Lee et al., 2008; Ullah et al., 2010). Clinical and epidemiologic studies have shown an association between hypertension and vD status. In NHANES III, a significant inverse association was reported between serum 25(OH)D concentration and blood pressure after adjusting for age, gender, ethnicity and physical activity, and was most marked among non-Hispanic blacks (Scragg et al., 2007). The adjusted prevalence of hypertension in U.S. adults was 30% higher in the lowest compared to the highest quintile of 25(OH)D concentration and that 25(OH)D levels were significantly lower in women, the elderly, ethnic minorities and persons with obesity, hypertension and diabetes mellitus (Martins et al., 2007). Prospective reports from the Health Professionals’ Follow-up Study and the Nurses’ Health Study also supported an association between vD deficiency and hypertension (Forman et al., 2007).

In clinical studies, increased blood vD levels are associated with reduced blood pressure (Krause et al., 1998). A randomized placebo-controlled study of 145 elderly women showed that 800 IU vitamin D3 plus 1200 mg of calcium significantly reduced blood pressure by 9.3% after 8 weeks, whereas treatment with 1200 mg calcium alone reduced blood pressure by only 4% (Pfeifer et al., 2001). However, no association was found between vD intake from diet and supplements and the risk of incident hypertension in two large cohort studies (Forman et al., 2005; Margolis et al., 2008). Skin pigmentation with melanin can reduce solar UV-B mediated cutaneous synthesis of vD by as much as 99%, which may contribute to the higher prevalence of vD deficiency in African Americans (Clemens et al., 1982). Systolic and diastolic blood pressures are significantly and positively associated with distance from the equator (Rostand, 1997).
Interactions between Vitamins A and D

vA and vD are inversely related and mutually inhibitory; hence, the effects of each one depend partly on the intake or availability of the other (Johansson and Melhus, 2001). In rats and humans, both retinyl acetate and retinoic acid inhibit the rise in serum calcium induced by vD (Rohde and DeLuca, 2005). Animal experiments have shown that vA decreases the toxicity of vD and increases the dietary need for vD (Melhus et al., 1998). Conversely, dietary vD from cod liver oil and butterfat protects against vA toxicity and allows for higher amounts of vA to be consumed before the latter becomes toxic (Myhre et al., 2003; Aburto et al., 1998). Clinical studies on hypervitaminosis A have shown that supplementation with vD reduces the toxicity of vA (Myhre et al, 2003), while high intakes of vA may worsen the effect of hypovitaminosis D (Johansson, 2004). The toxicity of vA and vD may therefore depend on the relative amounts or degree of imbalance between the vitamins rather than on the amounts of each vitamin considered in isolation (Masterjohn, 2009). With regard to the mechanisms underlying the reciprocal actions of vA and vD, high levels of vD reportedly lower vA stores in the liver and reduce serum vA concentrations (Aburto et al., 1998). Exposure of chickens to UV light, which produces vD, reduces liver stores and blood levels of vA (Aburto and Briton, 1998). Retinoid receptors bind retinoids in the form of dimers, as homodimers (RXR/RXR) or heterodimers (RAR/RXR), and heterodimers can also be formed between RXR and the vD receptor (VDR/RXR). RXR selective retinoids may therefore influence vD target genes in addition to the RXR genes, while vD may regulate RXR responsive genes. Combinations of RXR selective retinoids and vD derivatives may potentiate the expected therapeutic result and reduce the toxicity of each compound (Carlberg and Saurat, 1996; Reichrath et al., 2007).

Significance

There is some evidence that serum concentrations of retinol and RBP are associated with hypertension and associated risk factors for CVD but little evidence to date that dietary and/or supplementary intake of vA are associated with these outcomes; indeed, the reverse appears to be true. In the case of vD, there is again little evidence that dietary intake is associated with hypertension, but stronger evidence of an association between low serum vD concentration and hypertension. An inverse and mutually inhibitory relationship also exists between vA and vD such that, at the extremes, a primary deficiency of vD may increase the toxic effects of vA and high concentrations of vD inhibit the effect of vA; conversely, high concentrations of vA may reduce the actions of vD. Within the range of usual dietary intakes, relatively high intakes and/or concentrations of vA and vD may moderate the toxic effects of the other. Our study will assess the separate and combined impact of dietary intakes of vA and vD on blood pressure and hypertension in the context of a large prospective study on the development of heart disease. Future studies are in preparation by the investigators on interactions between serum concentrations of vA and vD in hypertension and CVD.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

Methods

This study will have 2 components, cross-sectional and longitudinal. For the cross-sectional analyses, we will analyze, by race, the association between dietary vitamin A/vitamin D intake at visit 1 and at visit 3 and each of the following:
1. mean systolic and diastolic BP;
2. the prevalence of hypertension;
3. the prevalence of CVD.

For the longitudinal analyses, we will analyze, by race, the association between dietary vitamin A/vitamin D intake at visit 1 and each of the following outcomes:
1. incidence of hypertension;
2. incidence of CVD events;
3. CHD mortality;
4. All-cause mortality.

The following participants will be excluded: individuals whose self-described race is neither black nor white (due to their small numbers); African American participants in the Minneapolis and Washington County centers (due to their small numbers); individuals with incomplete dietary information; and persons with diabetes (because diabetics tend to change their diets over time). In the longitudinal study, when incident hypertension is the outcome, individuals with prevalent CHD/stroke at baseline will also be excluded, as well as individuals with systolic BP =140 mmHg or higher and/or diastolic BP =90 mmHg or higher and/or persons using antihypertensive medication at baseline.

The main exposure variables will be dietary retinol/vitamin A intake and dietary vitamin D intake, each categorized in sex-specific quintiles. Usual dietary intake during the previous year was evaluated by a semi-quantitative food frequency questionnaire at ARIC Visit 1 (1987-1989, baseline) and Visit 3 (1993-1995). This was a 66-item questionnaire modified from Willett’s questionnaire. The main modifications were: 1) The questionnaire was administered by an interviewer using several measuring cups to help with portion size estimation, rather than being self-administered; 2) the addition of questions about fish consumption; 3) the re-organization of questions about vitamins and alcohol; 4) the addition of questions about cooking fats; and 5) the addition of open categories for frequently consumed foods. Nutrient intake was estimated using the nutrient database and software provided by Dr. Willett and adapted for the ARIC Study.

Other dietary variables that will be considered as covariates because of their potential role as dietary determinants of hypertension are: calcium, potassium, magnesium, sodium, alcohol and caloric intake. Variables that will be considered as potential confounders/effect modifiers/intermediate factors, in addition to the dietary factors, include age, sex, race, study center, socioeconomic status (i.e., educational attainment), kidney function (eGFR), uric acid level, blood lipid levels, physical activity, and BMI.

**Data analysis**
For the cross-sectional analyses we will run multiple linear regression models with quintiles of dietary vitamin A/vitamin D intake as the main independent variables and systolic or diastolic BP as the dependent variables. Additional contingency table analyses will consider the prevalence of hypertension and CVD as categorical dependent variables. For the longitudinal analyses, Cox proportional hazards regression models will be run, with time-to-diagnosis of hypertension/CVD or time-to-death as the main outcomes, and taking into account censoring of individual observations. A first model will include age, sex, and race/study center. A second
model will include known non-dietary risk factors for hypertension/CVD (e.g., education, cholesterol levels, BMI, physical activity, eGFR, uric acid). A third model will include other dietary factors.

References


DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 2004;80:1689S-96S.


Rostand SG. Ultraviolet light may contribute to geographical and racial blood pressure differences. Hypertension 1997; 30:150-156.


7.a. Will the data be used for non-CVD analysis in this manuscript? ___Yes  X No

8.a. Will the DNA data be used in this manuscript?  ___Yes  X No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/ARIC/search.php ___X___ Yes ___ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ___Yes  X No

12. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.