Materials and Methods

Materials and Methods are available in the online-only Data Supplement

Study population and protocol

The "SUpplementation en VItaminesetMinerauxAntioXydants" (SU.VI.MAX) study is a randomized double-blind, placebo-controlled, primary-prevention trial which started in 1994 in France. This epidemiologic study is designed to test the efficacy of a daily supplementation with antioxidant vitamins and minerals at nutritional doses, in reducing the main causes of premature death (cancers and cardiovascular diseases)\(^1\).\(^2\). In 2006, about 7200 individuals have agreed to be followed-up in the SU.VI.MAX – 2 study, an additional follow-up study, with the aim of exploring the associations between diet and aging in France. A specific sub-protocol was designed to assess microcirculation parameters in participants of the SU.VI.MAX – 2 cohort living in the Paris area. A total of 291 individuals without a history of type 2 diabetes (defined as fasting glucose>1.25 g/l and/or taking diabetes medication), hypertension (defined as BP<140/90mmHg and/or taking antihypertensive medication) and cancer or cardiovascular disease (CVD) events since 1994 was selected and included in the present study. Individuals were studied at the Department of Physiology and Noninvasive Investigations (Hospital Lariboisiere, Paris) between November 2006 and July 2007. The SU.VI.MAX and SU.VI.MAX – 2 studies were conducted according to the Declaration of Helsinki guidelines and were approved by the Ethics Committee for Studies with Human Subjects of Paris-Cochin Hospital (CCPPRB n° 706 and n° 2364, respectively) and the Comité National InformatiqueetLiberté (CNIL n° 334641 and n° 907094, respectively). Written informed consent was obtained from all participants.

Microvascular assessment

Capillaroscopy was carried out using a standardized validated technique detailed elsewhere\(^3\)\(^-\)\(^5\). Briefly, individuals were studied between 8:00 and 12:00 hr after an overnight fast. They were asked not to smoke from the previous evening. The capillaroscopy studies were performed in a temperature-controlled quiet room (21–24°C) after a10-min rest in the semisupine position. Patients were seated with the forearm and hand supported at heart level. The skin of the dorsum of the middle phalanx of the dominant hand was examined. Video microscopy was performed with an epi-illuminated optic fiber microscope containing a 100-W mercury vapor lamp light source and a M200 objective (Moritex, micro-ScopernanMS-500C, Tokyo, Japan); final x200 magnification was used. An approximately 3x3 mm skin area on the middle third of the phalanx was defined. Four microscopic fields (1mm\(^2\) each) were randomly chosen in this area for recording and measurements. Mean capillary density was defined as the number of capillaries per unit area of skin and was calculated as the mean of the four measurements performed in each individual. For each individual, images were acquired at baseline, to quantify the total number of continuously erythrocyte-perfused capillaries per dermal surface unit (resting or functional capillary density), and during venous congestion (by applying a cuff to the wrist and maintaining a 50-mmHg inflating pressure for 2 min), in order to obtain the maximal response of all existing capillaries and to assess structural (anatomical) capillary density. Indeed, it has been shown that this procedure maximizes visible capillary number more than reactive hyperemia\(^6\). A previous study\(^4\) has shown that intra-observer and inter-observer repeatability was 4.3 and 5.9%, respectively. Capillary recruitment (%) was defined as follows: [(capillary density during venous occlusion – resting capillary density)/resting capillary density] \* 100.

Hemodynamic anthropometric and anthropometric measurements
Brachial blood pressure (BP) and heart rate (HR) were measured in the sitting position with a semi-automatic oscillometric device (Dinamap PRO 400V2; General Electric, Fairfield, Connecticut, USA) with appropriate cuff size. After 10 min of rest, two measurements in each arm were obtained at 5-min intervals. SBP and DBP were calculated as the mean of the left and right second measurements. Mean arterial pressure (MAP) was calculated as: (DBP) + (SBP-DBP)/3. Peripheral pulse pressure (PP) was defined as the difference between the values of brachial systolic and diastolic pressures. Body weight and body composition were measured using the Tanita DC-320 (Tanita Corp., Tokyo, Japan) bioelectrical impedance device based on four separate footpad electrodes mounted on the system’s base. All measurements were carried out at 50 kHz with a 0.8 mA, with individuals in indoor clothing and no shoes.

Biochemical measurements

Serum total cholesterol was measured using standard methods. Fasting plasma glucose was assayed enzymatically (hexokinase) using a multiparametric analyzer (C8000 Architect Abbott analyzer, Rungis, France). Fasting plasma insulin was measured by microparticle enzyme immunoassay (Axsym Abbott analyzer, Rungis, France). Insulin resistance was estimated by the calculation of the homeostasis model assessment-insulin resistance (HOMA-IR) index (fasting plasma insulin * fasting plasma glucose) / 22.5.

Dietary data assessment

During the SU.VI.MAX 2 Study, subjects were invited to complete a validated food frequency questionnaire (FFQ) which assessed consumption of 250 food and beverage items during past year. Subjects were asked to report their consumption frequency the last 12 months, on the basis of how many times they ate the standard portion size proposed (photographs, typical household measurements such as spoon or standard unit such as a yogurt). Daily nutrient intakes were calculated using a composition table.

Information on smoking status (never smoked, former or current smoker), physical activity (irregular, equivalent to less than 1h of walk per day, equivalent to at least 1h of walk per day) and education (primary, secondary or university level) was collected using administered questionnaires.

Statistical analysis

Dietary pattern extraction

Data were analyzed using Statistical Package for Social Sciences software (version 13.0, 2004, SPSS Inc, Chicago, IL). Dietary patterns from 40 food groups were identified using principal components analysis (PCA). The Kaiser-Meyer-Olkin (KMO) criterion was applied and it was equal to 0.694. Derivation of optimal non-correlated components (dietary patterns) was performed using the orthogonal rotation (varimax option). Factor loadings represent the correlations of each food or food group with the dietary pattern score. Higher absolute values of factor loadings indicate that the food or food group predictor contributes most to the construction of this particular component. The dietary components (patterns) were named according to the factor loadings of those foods or food groups correlated most with the component (factor loadings > |0.4|). Dietary components derived from PCA were also confirmed using confirmatory factor analysis (CFA) with Stata statistical software release 12 (StataCorp LP 2011, TX, USA).
T-test or chi-square test was applied for the comparison of the population's characteristics between men vs. women. Multiple linear regression analysis was used to evaluate the independent associations between functional capillary density, anatomical capillary density and capillary recruitment with dietary patterns derived from PCA which are treated as continuous variables. More specifically, 3 different models were applied: model 1: adjusted for age, gender, BMI and energy intake, model 2: model 1 plus smoking, educational level, physical activity, HOMA-IR. The results from the linear regression models are presented as standardized beta coefficients and the level of significance was defined as a P<0.05. Moreover, participants’ dietary pattern scores were categorized into tertiles so that for each dietary component, tertile 3 consisted of persons whose dietary intake was most adherent to that particular pattern. Based on the statistical significant associations provided by the linear regression models, logistic regression analysis was performed to evaluate the association between the tertiles of each dietary pattern and the highest quartile of microcirculation indices. The results of logistic regression models were presented as odds-ratios (OR) and 95% confidence intervals (CI).

References