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Association of consumption of dairy products and meat with retinal vessel calibers in subjects at increased cardiovascular risk

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Abstract *Background and aims:* Association of dairy products and meat consumption with macrocirculation is previously described, but such association with microcirculation is poorly investigated. We aimed to test the hypothesis that the consumption of high- and low-fat dairy products as well as red, white, and processed meat is associated with retinal vessel calibers in adults at an increased risk of cardiovascular disease (CVD).

Methods and results: In consecutive subjects ($n = 181$, age: 51.3 ± 12.4 years, 51.4% women) without CVD and diabetes mellitus but with increased CVD risk, we obtained digital left and right retinal images. These images were assessed with validated software to determine central retinal arteriolar and venular equivalents and the arteriolar to venular ratio (CRAE, CRVE, and AVR, respectively). The consumption of dairy products and meat was assessed through 24-h recalls in all volunteers. After adjustment for potential confounders, the following findings were obtained: (i) low-fat milk and yogurt were positively associated with CRAE ($b = 0.145$, $p = 0.031$ left; $b = 0.141$, $p = 0.038$ right) and inversely associated with CRVE ($b = -0.155$, $p = 0.026$ left; $b = -0.146$, $p = 0.041$ right); (ii) low-fat cheese was positively associated with CRAE ($b = 0.164$, $p = 0.011$ left and $b = 0.155$, $p = 0.017$ right); and (iii) red meat was inversely associated with CRAE ($b = -0.143$, $p = 0.032$ left; $b = -0.114$, $p = 0.050$ right). High-fat milk, yogurt, and cheese or white and processed meat were not found to be associated with retinal vessel calibers.

Conclusions: High consumption of low-fat milk, yogurt, and cheese and low consumption of red meat could be beneficial for retinal microvascular health. Prospective studies are needed to verify these findings.

Introduction

Studies conducted in the past decades have shown that daily consumption of dairy products is inversely associated with the risk of metabolic syndrome, hypertension, and cardiovascular disease (CVD) [1,2]. Poor calcium intake, attributed to poor dairy intake, has also been proposed as a risk factor for many diseases including, but not limited to, hypertension and CVD [2,3]. On the contrary, high

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consumption of meat, especially red and processed meat, has been implicated in the increased risk of metabolic syndrome and hypertension [4,5], whereas most international guidelines recommend reducing red meat consumption to reduce CVD risk [6]. Only limited data have been obtained on the association of nutrients with impaired and/or damaged microcirculation [7,8], providing a potential link between dietary preferences and the genesis of metabolic and hemodynamic disorders, as well as CVD.

The retinal microvasculature offers an easily accessible site to evaluate the microcirculation and measure the retinal vascular caliber in a noninvasive manner for additional information on cardiovascular risk; however, its clinical utility remains unclear [9,10]. The retinal microvasculature is closely associated with CVD risk and disease [9]. In previous studies, wider retinal venules were found to be associated with incident CVD, and narrower retinal arterioles with incident coronary heart disease and hypertension in older adults [11]. Very few studies have investigated the association between diet and retinal microcirculation. These population-based studies, conducted in children and adults, showed that a high-glycemic-index diet and low consumption of fish and fiber diet are associated with unfavorable retinal microvascular health [7]. Moreover, recent results from the Blue Mountain Eye Study in healthy adults revealed an inverse association of low dairy consumption and high intake of low-fat dairy products and calcium with retinal microvascular health [12]. In addition, the results from the Sydney Childhood Eye Study showed a positive association of yogurt consumption with retinal arteriolar caliber in adolescents, but significant associations were not observed with the intake of other dairy products [13]. To the best of our knowledge, studies relevant to meat consumption and retinal microcirculation have not been conducted.

The aim of the present cross-sectional study was to investigate the possible associations of high- and low-fat dairy product intake, as well as red, white, and processed meat consumption, with retinal vessel calibers in individuals at increased CVD risk (free of diabetes and established CVD), treated for the presence of CVD risk factors.

Methods

Study design: population

Consecutive adults at increased CVD risk due to the presence of classical or novel (chronic inflammatory diseases) CVD risk factors were systematically referred to the Cardiovascular Research Laboratory of our department for optimal CVD risk stratification and were included in the study. Participants with established CVD (defined as preexisting coronary artery disease, stroke, and peripheral arterial disease) and diabetes mellitus were excluded from the analysis. All participants underwent the same vascular investigations, and they provided written informed consent according to the World Health Organization statement on ethical principles for medical research involving human subjects developed in Helsinki. Further, the protocol was approved by the "Laiko" Hospital's institutional review board.

Definition of CVD risk factors

Hypertension was defined as the use of antihypertensive drugs and/or office blood pressure measurement >139/89 mmHg (average of three sequential readings with 1-min interval in the supine position after at least 10 min of rest; Microlife WatchBP Office, Microlife AG, Widnau, Switzerland). Dyslipidemia was defined by treatment with lipid-modifying drugs or low-density lipoprotein cholesterol level >160 mg/dl. Current smoking was defined by the use of at least one cigarette per day each day of the week; ex-smoking was defined as discontinuation of smoking for >6 months. Body mass index was calculated as weight/(height [2]) (kg/m^2), which was used as a marker of obesity. A family history of premature CVD was defined as the presence of coronary heart disease in a first-degree relative below the age of 55 years for men and 65 years for women. Required data were retrieved from the medical records of the participants.

Dietary assessment

Dietary intake was evaluated through two 24-h recalls collected over the phone by a trained dietician. The study participants were asked to recall and report to the dietician the type and amount of foods and beverages consumed the previous 24 h; this procedure was performed for one weekday and one weekend day (nonconsecutive days). Data from the recalls were analyzed for their energy, macronutrient, and selected micronutrient content via Nutritionist Pro, version 5.2 software (Axxya Systems-Nutritionist Pro, Stafford, TX, USA). The Nutritionist Pro food database was expanded by adding analyses of traditional Greek foods and recipes. For the present analysis, we used dairy and meat food groups, as well as the following subgroups: regular milk and yogurt, low-fat milk and yogurt, regular cheese, low-fat cheese, red meat, white meat, and processed meat. Serving sizes of 250 ml were used for milk, one cup for yogurt, 30 g for cheese, 60 g for red and white meat, and 30 g for processed meat. The above-mentioned servings were selected according to the food-based dietary guidelines for Greece (http://www.fao.org/nutrition/education/food-based-dietary-guidelines/regions/countries/greece/en/), to better reflect portion sizes used by a Greek population. The daily consumption of the above-mentioned subgroups in our population is presented in Table 1.

Analysis of retinal vasculature

Both eyes of each participant were photographed with a 45° digital non-mydriatic retinal camera (Topcon TRC-NW8, Tokyo, Japan) after 5 min of adapting to the dark using a validated method [14]. Retinal images were

Table 1 General characteristics of study participants.

N	Total
	181
Age (years)	51.3 ± 12.4
Women (%)	51.4
Body mass index (kg/m^2)	27.1 ± 5.1
Smoking (%)	58
Hypertension (%)	39.8
Dyslipidemia (%)	26
Chronic disease (%)	49.4
Peripheral systolic blood pressure (mmHg)	123.6 ± 16.7
Peripheral diastolic blood pressure (mmHg)	76.4 ± 10.4
CRAE, Left (μm)	176.0 ± 17.0
CRAE, Right (μm)	176.8 ± 16.8
CRVE, Left (μm)	211.0 ± 17.3
CRVE, Right (μm)	211.8 ± 17.5
AVR, Left	0.84 ± 0.07
AVR, Right	0.84 ± 0.07
Daily Kilocalories (Kcal)	1937 ± 764.3
Low-fat milk and yogurt (servings/day)[a]	0.4 ± 0.6
High-fat milk and yogurt (servings/day)[a]	0.2 ± 0.4
Low-fat cheese (servings/day)[b]	0.2 ± 0.4
High-fat cheese (servings/day)[b]	1.1 ± 1.2
Red meat (serving/day)[c]	1.1 ± 1.0
White meat (serving/day)[c]	0.5 ± 0.8
Processed meat (serving/day)[b]	0.4 ± 0.7

CRAE: central retinal arteriolar equivalent, CRVE: central retinal venular equivalent, AVR: arteriolar to venular ratio. Values are presented as mean ± standard deviation or percentage (%).
[a] 250 ml of milk, or one cup of yogurt.
[b] 30 g.
[c] 60 g.

centered on the optic disc, followed by quantitative retinal grading, conducted by a well-trained physician (EKA) blinded to the clinical data. For each photograph, the calibers of retinal arterioles and venules passing through a zone extending from 0.5 to 1.0 disc diameters from the optic disc margin were measured and analyzed using a Static Retinal Vessel Analyzer (SVA-T and Vesselmap 2 software, Visualis, Imedos Systems UG, Jena, Germany) [14]. These measurements were then summarized using formulas described elsewhere [15] to compute the central retinal arteriolar equivalent (CRAE) and the central retinal venular equivalent (CRVE), representing the average internal caliber of retinal arterioles and venules, respectively. In addition, CRAE and ARVE were used to estimate the arteriolar to venular ratio (AVR). The intra-observer reproducibility of retinal vascular measurements was excellent (intraclass correlation coefficients >0.93).

Statistical analysis

Statistical analysis was performed using the SPSS statistical package (IBM, version 21.0; IBM, Armonk, NY, USA). The normality of the variables was tested using the Kolmogorov−Smirnov test and histograms. Normally distributed variables are presented as mean values (standard deviation) and categorical variables as frequencies. Multiple linear regression (enter method) was performed to determine the relationship of high- and low-fat dairy products, as well as red, white, and processed meat, with retinal vessel calibers, while adjusting for potential covariates [16]. The potential confounders were selected based on previous publications [16] and among variables that showed univariate analysis association ($p < 0.10$) with retinal vessel calibers in the present study. The models testing CRAE and CRVE related to food items were additionally adjusted for fellow caliber to provide unbiased and biologically plausible results as suggested by Liew et al. [17]. The results are presented as beta coefficients and *p*-values. All tests were two sided, and the level of statistical significance was set at $p < 0.05$.

Results

The baseline characteristics of the study population and mean caloric intake as well as mean intake of low- and high-fat milk, yogurt, and cheese, as well as red, white, and processed meat, are summarized in Table 1.

Table 2 presents the associations derived from linear regression analysis between the consumption of low-fat milk and yogurt or low-fat and cheese indices and retinal vessel calibers. After adjustment for potential confounders, the following were observed: (i) high consumption of low-fat milk and yogurt were positively associated with CRAE of both eyes ($b = 0.145$, $p = 0.031$ for left eye, and $b = 0.141$, $p = 0.038$ for right eye, respectively) and AVR of both eyes ($b = 0.210$, $p = 0.012$ for left eye and $b = 0.194$, $p = 0.022$ for right eye, respectively); (ii) consumption of low-fat milk and yogurt was inversely associated with CRVE of both eyes

Table 2 Associations between daily consumption of low-fat milk and yogurt, or low-fat cheese and retinal vessel calibers.

Components	Model 2	
	β	*p*-value
Low-fat milk and yogurt		
CRAE (Left) μm	**0.145**	**0.031**
CRAE (Right) μm	**0.141**	**0.038**
CRVE (Left) μm	**−0.155**	**0.026**
CRVE (Right) μm	**−0.146**	**0.041**
AVR (Left)	**0.210**	**0.012**
AVR (Right)	**0.194**	**0.022**
Low-fat cheese		
CRAE (Left) μm	**0.164**	**0.011**
CRAE (Right) μm	**0.155**	**0.017**
CRVE (Left) μm	−0.101	0.140
CRVE (Right) μm	−0.066	0.347
AVR (Left)	**0.184**	**0.022**
AVR (Right)	**0.161**	**0.049**

CRAE: central retinal arteriolar equivalent, CRVE: central retinal venular equivalent, AVR: arteriolar to venular ratio.
Model 2: adjusted for age, gender, hypertension, smoking, hyperlipidemia, family history of CAD, existence of autoimmune disease, drugs for hyperlipidemia and hypertension, kilocalories, CRAE, and CRVE (in accordance with the dependent variable).
β: standardized beta coefficient.

($b = -0.155$, $p = 0.026$ for left eye and $b = -0.146$, $p = 0.041$ for right eye, respectively); and (iii) consumption of low-fat cheese was positively associated with CRAE ($b = 0.164$, $p = 0.011$ for left eye and $b = 0.155$, $p = 0.017$ for right eye, respectively) and AVR ($b = 0.184$, $p = 0.022$ for left eye and $b = 0.161$, $p = 0.049$ for right eye, respectively).

No association was noted between the consumption of high-fat milk and yogurt, or high-fat cheese, and retinal vessel calibers in all models and after adjustment for several confounders, which is presented in Table 3.

Table 4 presents an inverse association between high consumption of red meat and CRAE ($b = -0.143$, $p = 0.032$ for left eye and $b = -0.114$, $p = 0.050$ for right eye, respectively) and AVR in the left eye ($b = -0.174$, $p = 0.037$) after adjustment for potential confounders. No association was found between the consumption of white or processed meat and retinal vessel calibers.

We also performed an additional regression analysis for the association of calcium intake with indices of retinal microcirculation. Calcium intake was found to be inversely associated with CRVE ($b = -0.186$, $p = 0.023$) and positively with AVR of the right eye ($b = 0.198$, $p = 0.042$) after adjusting for all potential confounders (model 2) (data not shown in tables).

Table 4 Associations between daily consumption of red meat, white meat, or processed meat and retinal vessel calibers.

Components	Model 2	
	β	*p*-value
Red meat		
CRAE (Left) μm	**−0.143**	**0.032**
CRAE (Right) μm	**−0.114**	**0.050**
CRVE (Left) μm	0.065	0.352
CRVE (Right) μm	0.057	0.426
AVR (Left) μm	**−0.174**	**0.037**
AVR (Right) μm	−0.135	0.111
White meat		
CRAE (Left) μm	0.058	0.399
CRAE (Right) μm	0.019	0.726
CRVE (Left) μm	0.070	0.325
CRVE (Right) μm	0.091	0.214
AVR (Left) μm	0.022	0.799
AVR (Right) μm	−0.020	0.818
Processed meat		
CRAE (Left) μm	−0.030	0.648
CRAE (Right) μm	−0.017	0.794
CRVE (Left) μm	−0.003	0.960
CRVE (Right) μm	−0.012	0.868
AVR (Left) μm	−0.039	0.630
AVR (Right) μm	−0.020	0.807

CRAE: central retinal arteriolar equivalent, CRVE: central retinal venular equivalent, AVR: arteriolar to venular ratio.
Model 2: adjusted for age, gender, hypertension, smoking, hyperlipidemia, family history of CAD, existence of autoimmune disease, drugs for hyperlipidemia and hypertension, kilocalories, CRAE, and CRVE (in accordance with the dependent variable).
β: standardized beta coefficient.

Discussion

This cross-sectional study investigated the associations between dietary intake – including dairy foods, and red, white, and processed meat – with retinal vessel diameters in adults at high CVD risk. The results showed that the consumption of low-fat milk, yogurt, and cheese was positively associated with retinal arteriolar caliber and inversely associated with retinal venular caliber. In addition, red meat consumption was inversely associated with retinal arteriolar caliber.

Table 3 Associations between daily consumption of high-fat milk and yogurt, or high-fat cheese and retinal vessel calibers.

Components	Model 2	
	β	*p*-value
High-fat milk and yogurt		
CRAE (Left) μm	−0.047	0.470
CRAE (Right) μm	−0.032	0.629
CRVE (Left) μm	0.017	0.806
CRVE (Right) μm	−0.021	0.760
AVR (Left) μm	−0.039	0.628
AVR (Right) μm	−0.024	0.768
High-fat cheese		
CRAE (Left) μm	0.024	0.717
CRAE (Right) μm	0.026	0.705
CRVE (Left) μm	−0.004	0.954
CRVE (Right) μm	−0.045	0.532
AVR (Left) μm	0.027	0.749
AVR (Right) μm	0.048	0.572

CRAE: central retinal arteriolar equivalent, CRVE: central retinal venular equivalent, AVR: arteriolar to venular ratio.
Model 2: adjusted for age, gender, hypertension, smoking, hyperlipidemia, family history of CAD, existence of autoimmune disease, drugs for hyperlipidemia and hypertension, kilocalories, CRAE, and CRVE (in accordance with the dependent variable).

Only two previous studies investigated the associations of dairy products with retinal microvasculature. The first study [13] reported that high consumption of yogurt is associated with wider retinal arterioles and narrower retinal venules. However, this study assessed total dairy consumption instead of high- and low-fat dairy consumption, which was the aim of the present study, rendering any comparison difficult. The second study also assessed total dairy consumption, finding an inverse association with retinal venular caliber [12]. Interestingly, this study also assessed the possible associations of low-fat dairy products (including milk, dairy sweets, and cheese) with retinal microcirculation. The results showed that participants in the lowest quintile of consumption of low-fat dairy products had wider retinal venules and narrower retinal arterioles. These findings are in line with the present results, which revealed that the consumption of low-fat milk and yogurt and low-fat cheese was positively associated with retinal arteriolar calibers and AVR, and that the consumption of low-fat milk and yogurt was inversely associated with retinal venular calibers. Several future studies should investigate these associations in depth. However, it is of note that the same outcome regarding the associations of low-fat dairy product consumption with retinal microcirculation was observed in

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different adult populations (healthy adults and those with CVD risk factors in the present study).

One possible explanation for the present findings is the presence of saturated fat in high-fat dairy products. However, in the present study, no association was found between high-fat milk, yogurt, and cheese and retinal microcirculation, which suggests that constituents of dairy products other than fat may be responsible for the observed results. Some studies suggest that bioactive peptides in milk and cheese may inhibit the renin–angiotensin system, reducing blood pressure levels [18], whereas others show that peptides found in dairy products exert significant antioxidant effects, which act beneficially in endothelial function [19]. Furthermore, calcium has been reported to play a protective role via its effects on reducing hypertension and inflammation levels [23]. In our study, calcium was found to be independently associated with improved retinal microvasculature, as high consumption was associated with narrower venular retinal calibers. Calcium in combination with other important components of dairy products may explain the observed results in retinal microcirculation.

Regarding meat consumption, an inverse association was noted between high consumption of red meat and retinal arteriolar caliber, as well as AVR in both eyes. In addition, there was no association between consumption of either white or processed meat and retinal microvasculature. High consumption of red meat is considered to increase CVD risk [24], which could be partially explained by its effects on microcirculation. To our knowledge, the possible effects of meat consumption on retinal microcirculation have not been investigated yet. In the European Eye Study (EUREYE), one of the dietary patterns extracted a posteriori from this population was characterized as "unhealthy" and included red and processed meat consumption, together with refined grains, eggs, butter, and sweets [25]. However, no association was shown between this dietary pattern and retinal microcirculation. Further, the SU.VI.MAX 2 study, which assessed microcirculation in the skin instead of the retina, revealed a dietary pattern including red and processed meat together with high-fat sauces, refined grain products, and spices [8]. Similarly, this dietary pattern had no association with skin microcirculation. However, dietary patterns present a group of dietary preferences instead of a single food item, and their associations with clinical outcomes represent the synergistic effect of those foods, rather that the direct effect of one of their components [26].

In our population, the consumption of white and processed meat was very low, which might have obscured an association with retinal microcirculation. However, the observed inverse association of red meat consumption with retinal arteriolar caliber and AVR remained significant in all multivariate models. Red meat is a rich source of both saturated fatty acids and cholesterol, which are reported to lead to an unfavorable lipid profile and to increased CVD risk [27]. Moreover, food preparation methods used for red meat (high-heat cooking) usually lead to the production of advanced glycation end products, which are highly oxidative compounds that enhance endothelial damage and dysfunction in the whole body and in the retinal microvasculature [30,31]. Nevertheless, several studies need to establish the possible association of meat consumption with retinal microcirculation, as well as to elucidate the underlying mechanisms.

The present study has some limitations. It is an observational study conducted in a relatively small and heterogeneous population. However, the presence of these associations in this type of population rather reinforces the validity of the results, which would be expected to be even stronger in homogeneous populations. We also acknowledge some limitations regarding the dietary assessment method. The assessment of habitual dietary consumption is a serious task. All available methods are based on self-reported data, possibly biased by under- or mis-reporting [32]. In line with previous European projects aiming to develop basic methods for dietary assessment for use in nutrition studies [34], we decided to use 24-h recalls of two nonconsecutive days for dietary assessment, to avoid dropouts and assessment bias. However, it should be acknowledged that the consumption of dairy products and meat may not be accurately estimated.

In conclusion, high consumption of low-fat milk, yogurt, and cheese and low consumption of red meat are associated with beneficial retinal microvascular patterns, even in individuals with known CVD risk factors. Further prospective longitudinal and intervention studies are required to additionally investigate the association between food types and their constituents with the microcirculation.

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