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Low plasma retinol predicts coronary events in healthy middle-aged men: The PRIME Study

K. Fred Gey[a,*,1,2], Pierre Ducimetière[b,1], Alun Evans[c,1], Philippe Amouyel[d,1], Dominique Arveiler[e,1], Jean Ferrières[f,1], Gerald Luc[d,1], Frank Kee[c,1], Annie Bingham[b,1], John Yarnell[c,1], François Cambien[g,1]

[a] The MONICA Reference Centre for Vitamins, Bern, Department of Biochemistry and Molecular Biology, University of Bern, Switzerland
[b] France MONICA Co-ordinating Centre, INSERM U258, Hôpital Paul Brousse, Villejuif, France
[c] The UKCRN Centre of Excellence for Public Health (NI), Queen's University of Belfast, UK
[d] Lille MONICA Centre, INSERM U508, Institut Pasteur de Lille, France
[e] Strasbourg MONICA Centre, Department of Epidemiology and Public Health, Medical School of Strasbourg, France
[f] Toulouse MONICA Centre, INSERM U558, Department of Epidemiology, Paul Sabatier-Medical School of Purpan, Toulouse, France
[g] INSERM U525, Université Pierre et Marie Curie, Paris, France

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ABSTRACT

The role of plasma retinol and carotenoids in coronary heart disease (CHD) remains unclear. The PRIME Study prospectively evaluated these in France and Northern Ireland in 9758 men aged 50–59 years who were free of CHD at baseline. After five years' follow-up 150 incident cases of CHD (non-fatal myocardial infarction and fatal CHD) were compared with 285 controls matched for age, date of blood collection and study centre. Geometric means of major carotenoids did not differ significantly between cases and controls ($P > 0.05$), whereas the absolute and lipid-standardized plasma retinol levels were 9% lower in cases than controls in both countries ($P < 0.002$), without correlation with carotenoids. After adjusting for risk factors, the relative risks (RRs) of CHD in the first four quintiles of retinol distribution in controls ($\leq$601, −683, −760, and −846 μg/l) were 2.65 ($P = 0.0009$), 1.70, 1.03, and 1.12 (all $P > 0.05$) respectively, relative to the top quintile (retinol $\geq$846 μg/l; linear trend $P = 0.0001$). The 10th percentile of lipid-standardized retinol ($\leq$544 μg/l) predicted an RR of 4.7 ($P < 0.001$). The risk associated with low retinol was comparable to strong risk factors (e.g. HDL-cholesterol, Interleukin-6) and behaved additively.

In conclusion, plasma retinol levels of <601 μg/l in a fifth of middle-aged European men place them at an approximately threefold RR of developing CHD. Thus the intake of vitamin A might be too low in middle-aged men. These findings must be confirmed.

1. Introduction

Low serum vitamin D has recently been shown to predispose to coronary heart disease (CHD), but whether vitamin A, another liposoluble vitamin with 'hormone-like' activity, is involved in atherosclerosis is unclear. This is plausible since retinoic acid, the bioactive form of vitamin A, regulates genes expression concerning cellular growth, differentiation and apoptosis, has antioxidative properties, participates in angiogenesis and reactivity of endothelial and smooth muscle cells, modulates cell interactions in inflammation [1–7], and can impair arterial re-stenosis [3,5].

We aimed to assess CHD risk associated with low levels of plasma carotenoids/vitamin A. The PRIME Study (prospective epidemiological study of myocardial infarction) in France and Northern Ireland provided the opportunity, thanks to striking differences in CHD incidence and lifestyle between the countries [6–8]. Plasma levels of vitamin A and carotenoid-precursors reflect bioactivity fairly accurately, for although 80–90% of vitamin A is stored in the liver, retinol is recycled extensively between plasma, liver and extrahepatic tissues according to retinoic acid needs [1,2,4].

2. Subjects and methods

2.1. Pooled study cohorts

From 1991 to 1993, 10,600 50–59-year old males, were recruited from the MONICA populations in Belfast, Northern Ireland, and Lille, Strasbourg and Toulouse in France [6–8]. Medical questionnaires included diseases affecting vitamin A status and consumption of

* Corresponding author. Present address: c/o Alun Evans, The UKCRN centre of Excellence for Public Health (NI), Queen's University of Belfast, Mulhouse Building, Grosvenor Road, Belfast BT12 6BJ, UK. Tel.: +44 28 9023 7153; fax: +44 28 9023 1907.
E-mail address: a.evans@qub.ac.uk (K.F. Gey).
[1] On behalf of the PRIME Study Group (see Appendix A).
[2] Formerly affiliation 'a'.

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fruit/vegetables, and vitamin supplements, over a typical week [9]. Written informed consent was obtained from all subjects. After 12-hours' fasting, heparinized plasma, prepared immediately from venous blood, was frozen within 15 min at −80 °C, and shipped weekly for liquid nitrogen storage at SERLIA, Lille [6–8].

According to Minnesota coding and history, 9758 men were CHD-free at baseline: 2399 in Northern Ireland and 7359 in France. After five years, 175 non-fatal MI or fatal CHD events, defined by MONICA criteria, included sudden coronary death (confirmed by clinical data/autopsy [7]): 69 in France (57 non-fatal MI, 14 coronary deaths), and 106 in Northern Ireland (89 non-fatal MI and 21 coronary deaths). Incident cases of stable and unstable angina were rejected as cases or controls. For each case, two controls were randomly selected from those free of CHD, at the time of the case's event, and matched for centre, age (±3 years), and date of examination (±3 days). Subjects consuming vitamins or 'tonics,' containing vitamins, at least weekly, were excluded. The few cases of diabetes precluded meaningful analysis. Full matching was possible for 167 cases and 334 controls, missing tubes/analyses reducing these to 150 cases (123 non-fatal MI; 27 CHD fatalities) and 285 controls: 58/111 in Northern Ireland, and 92/174 in France, respectively. To gain statistical power, pooled cases of CHD were compared with pooled controls.

2.2. Chemical analysis

In 2001, the plasma samples were blindly analysed by reverse-phase high-performance liquid chromatography [10] in the Swiss Reference Laboratory for Liposoluble Plasma Antioxidants (appointed by the National Institute of Standards & Technology, Gaithersburg, MD, after inter-laboratory calibration) and for triglycerides, total and HDL-cholesterol by automated standard assay. A random sample (n = 100) of all plasma samples, analysed after recruitment, and 6–7 years later, demonstrated no significant change in retinol or β-carotene (linear correlation coefficients >0.9).

Because absolute plasma retinol correlates with cholesterol and triglycerides, despite being carried by retinol-binding protein [1], lipid-standardized values are also presented using multiple linear regression to a cholesterol of 2.49 g/l (5.7 mmol/l) and triglycerides of 1.09 mg/l (1.25 mmol/l) [11]. A similar matching for inflammatory markers [8] provided an overlap for nearly all cases (n = 143) and controls (n = 242).

2.3. Statistics

Geometric means (keeping centre constant) were calculated for pooled cases and controls to adjust for statistically significant differences between centres: fruit/vegetables score, plasma HDL-cholesterol, carotenoids and retinol being higher in France, whereas alcohol intake, plasma total cholesterol, triglycerides and inflammation markers were higher in Northern Ireland. Univariate or multivariate comparisons of cases/controls (including interactions) were by unconditional logistic regression models. Interaction terms with specific covariates, examined seriatim, were introduced into the models. Covariates were dichotomized by their median distribution in controls, tobacco consumption excepted (smokers/non-smokers).

Skewed variables, i.e. triglycerides, high sensitivity C-Reactive Protein (hs-CRP), Interleukin-6 (IL-6), carotenoids, absolute and lipid-standardized retinol were log-transformed and geometric rather than simple means calculated. Correlations between variables were computed as Spearman's rank correlation coefficients for controls from each population, and as pooled cases versus controls. Relative risk (RR) of CHD for any variable was assessed for percentiles or standardized regression coefficients. Statistical significance denotes P < 0.05 unless otherwise stated. All P-values are two-tailed.

Table 1
Comparison of geometric means (keeping the centre constant) of pooled cases with non-fatal myocardial infarction or coronary death versus pooled controls at baseline: The PRIME Study.

	CHD cases (n = 150)	Controls (n = 285)	P
Body mass index (kg/m^2)	26.9	26.9	0.93
Smoking (cigarettes/day)[a]	**6.9**	**3.8**	**0.002**
Present smokers, %	**39.3**	**28.4**	**0.01**
Alcohol intake, ml/day	30.9	33.3	0.65
Fruit-vegetables consumption[b]	11.9	12.0	0.50
Systolic blood pressure, mm Hg	137.6	134.6	0.09
Diabetes, %	**8.0**	**2.1**	**0.008**
Total cholesterol, g/l	2.28	2.23	0.30
HDL-cholesterol, g/l	**0.43**	**0.48**	**0.0003**
HDL-cholesterol/total cholesterol	**0.19**	**0.22**	**0.0002**
Triglycerides, g/l[c]	1.48	1.35	0.09
Fibrinogen, g/l	3.62	3.43	0.06
hs-C-Reactive Protein[c]	**1.99**	**1.36**	**0.0002**
Interleukin-6[c]	**1.85**	**1.32**	**0.0001**
α-Carotene, μg/l[c]	40.5	42.7	0.57
β-Carotene, μg/l[c]	175.8	184.3	0.52
β-Cryptoxanthin, μg/l[c]	61.5	66.2	0.47
Lutein, μg/l[c]	118.2	121.4	0.51
Zeaxanthin, μg/l[c]	21.5	22.9	0.17
Lycopene, μg/l[c]	155.0	153.9	0.90
Total carotenoids, μg/l[c]	640.1	662.8	0.51
Retinol, absolute, μg/l[c]	**664.9**	**715.7**	**0.002**
Retinol, lipid-standard, μg/l[c]	**658.1**	**718.1**	**0.0001**

Statistical significance (p < 0.05) is indicated in bold.
[a] >1 cigarette daily.
[b] Arbitrary units.
[c] Log-transformed.

3. Results

3.1. Geometric means of retinol in cases and controls (Table 1) and correlations with fruit/vegetables and plasma carotenoids (Table 2)

At baseline, pooled cases had significantly higher levels (P < 0.05) of several classic risk factors than controls, e.g. cigarette smoking, HDL-cholesterol/total cholesterol, hs-CRP and IL-6, whereas no differences were observed (P < 0.06–0.09) for fibrinogen, systolic blood pressure, and triglycerides. Body mass index, total cholesterol, intake of alcohol, fruit/vegetables and all plasma carotenoids did not differ significantly between cases and controls (P > 0.1) despite fruit/vegetables score and all plasma carotenoids (except lycopene) being significantly lower in Northern Ireland than France (not shown). The fruit/vegetables score was positively associated with total carotenoids (partial Spearman correlation coefficient r = 0.34; P < 0.001) and all single carotenoids (r = 0.21–0.36; P < 0.001). The levels of individual carotenoids (except α-carotene) and their total were negatively associated with smoking (r = 0.10–0.22; P < 0.01–0.001) and positively with years of schooling: r = 0.16 and 0.20 in controls and cases respectively (P < 0.01).

The carotenoid-linked RRs for quintiles of the various carotenoids did not differ significantly and adjustment on classic risk factors had no effect, in either Northern Ireland, where carotenoids (except lycopene) were 27–54% lower (P mostly <0.0001), or France (not shown). In contrast, the geometric means of plasma levels of total and lipid-standardized retinol were respectively 665 and 658 μg/in cases compared to 716 and 718 μg/l in controls, i.e. 9% lower (P < 0.002), without interaction for country (P > 0.05).

Table 2
Partial Spearman correlation coefficients (holding region constant) of retinol and lipid-standardized retinol levels with risk factors in pooled controls (n = 285): The PRIME Study.

Potential risk factor for CHD	Absolute Retinol	Lipid-standardized Retinol
Age, years	−0.06	0.06
Fruit/vegetable score	0.03	0.06
Cigarette smoking	0.07	0.06
Alcohol intake	**0.21*****	**0.20*****
Systolic blood pressure	0.12*	0.10
Diabetes mellitus, %	0.00	0.03
Body mass index	−0.01	−0.04
Plasma total cholesterol	**0.21*****	0.01
HDL-cholesterol	0.08	**0.16****
HDL-cholesterol/total cholesterol	−0.08	**0.12***
Triglycerides	**0.30****	0.09
Fibrinogen	**−0.17****	**−0.16****
Hs-C-Reactive Protein	**−0.17****	**−0.21*****
Interleukin-6	−0.07	−0.10
β-Carotene	−0.04	0.01
β-Cryptoxanthin	−0.05	−0.05
Total carotenoids	0.01	0.03

Statistical significance ($p < 0.05$) is indicated in bold.
* Statistically significant correlation: $P < 0.05$.
** Statistically significant correlation: $P < 0.01$.
*** Statistically significant correlation: $P < 0.001$.

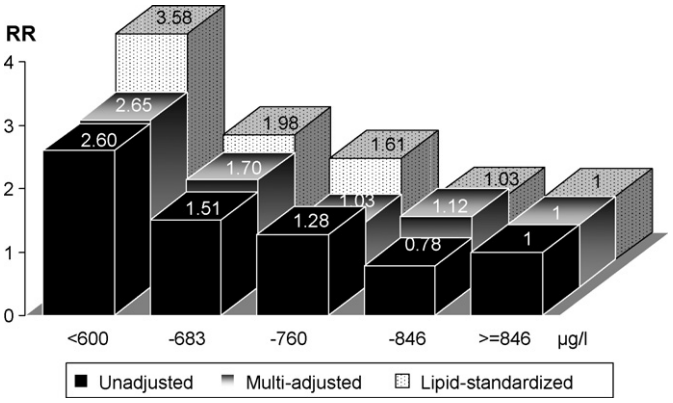

Fig. 1. Low plasma retinol (<601 µg/l, quintile 1) predicts significantly increased relative risk (RR) of CHD: the PRIME Study. Ordinates, and figures above all columns indicate centre-adjusted RRs in comparison with the reference status (quintile 5). *Abcissa*: quintiles in controls of plasma retinol in µg/l. *Front row*: unadjusted retinol; *Middle row*: multi-adjusted retinol (for systolic blood pressure, smoking, alcohol intake, diabetes, HDL- and total cholesterol, triglycerides, Body mass index, fibrinogen, hs-CRP, and log Interleukin-6). *Back row*: lipid-standardised retinol (to 220 mg/dl cholesterol and 110 mg/dl triglycerides). Statistical significance of columns of quintile 1 (<600 µg/l) ($P < 0.004$, 0.02, 0.004) and of the linear trend of increasing RR from quintile 5 to 1 ($P = 0.0001$).

3.2. Centre-adjusted correlations of plasma retinol with other variables (Table 2)

The pooled levels of absolute and lipid-standardized retinol varied independently of plasma carotenoids and fruit/vegetable intake in controls and cases, with identical correlation coefficients (not shown). In controls globally (Table 2), and in France and Northern Ireland separately (centre-specific analysis not shown), absolute and lipid-standardized retinols were significantly confounded by alcohol intake, without significant case/control differences. Plasma levels of fibrinogen and hs-CRP in cases and controls were positively correlated with absolute and lipid-standardized retinol ($P = 0.001$), but this was absent for IL-6. The weak positive association of retinol with cholesterol and triglycerides disappeared after lipid-standardization, but a positive correlation with HDL-cholesterol and HDL-cholesterol/total cholesterol ratio became significant.

3.3. CHD-related RR of low retinol (Figs. 1 and 2 and Table 3)

The RR of CHD increased with decreasing plasma retinol (Fig. 1). This obtained for the quintiles of absolute, lipid-standardized and multivariate-adjusted retinal—without significant centre differences. Taking the fifth quintile (>846 µg/l) as reference, the RR of quintiles 2 and 3 (602–760 µg/l) rose moderately (to 1.61- and 1.98-fold). The RR for quintile 1 (≤601 µg/l retinol) varied, depending on adjustment (see Fig. 1), between 2.60 ($P < 0.004$) and 3.58 ($P < 0.001$). The lowest decile (≤544 µg/l) predicted an RR of 3.58 (95% CI: 1.42–6.39) for crude retinol and 4.70 (95% CI: 2.21–9.97) after lipid-standardization ($P < 0.001$). Despite a significant linear trend ($Chi^2 = 4.8$, $P = 0.0001$), the RR in quintile 4 (761–846 µg/l) and the reference quintile 5 (≥846) did not differ significantly. They might be considered 'optimal' in terms of having the smallest RRs. The few subjects ($n = 3$) with retinol levels <400 µg/l (previously taken as borderline A-hypovitaminosis [12]) precluded analysis.

Quantifying the contribution of the significant risk factors in a continuous regression model (Table 3) indicated that a 1 SD increase in log retinol (0.224 in the control group) decreased the RR significantly by 29%, of lipid-standardized retinol by 35% in univariate analysis, and by 30% in unconditional logistic multivariate analysis. The effect size equated with that observed for an increase in HDL-cholesterol/total cholesterol ratio or an increase in IL-6 by 1 SD. The standardized RRs of these factors suggested the ranking: HDL-cholesterol/total cholesterol ratio ≈ IL-6 ≥ retinol ≥ smoking ≈ systolic blood pressure. In contrast total

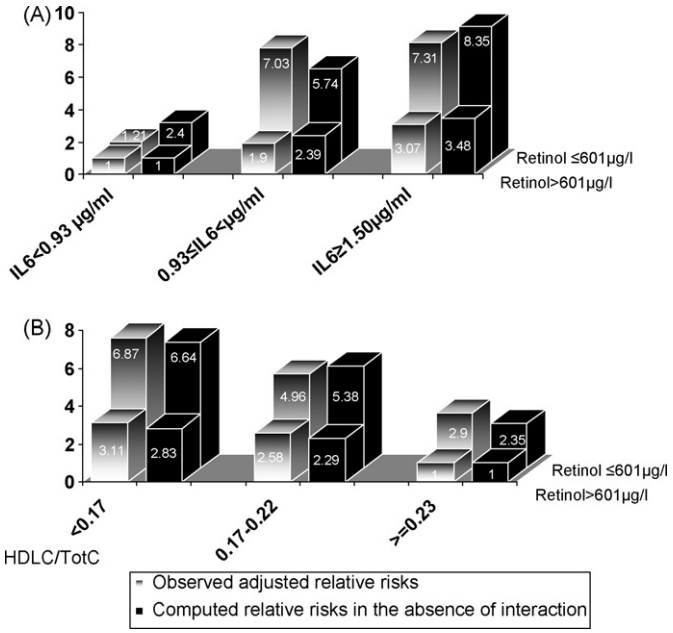

Fig. 2. Centre-adjusted RRs of retinol and of other risk factors for CHD behave additively but independently: the PRIME Study. Ordinates and figures within columns: RRs after multivariate adjustment for covariates as for column (c) of Fig. 1. *Front row*: low plasma retinol (<601 µg/l) associated with high RRs; *Back row*: higher retinol levels (>601 µg/l) associated with moderately low to no substantial RR. *Abscissae*: other risk factors in combination with plasma retinol: *Panel A*: tertiles of plasma Interleukin-6 (IL-6) in µg/ml with increasing risk gradient; *Panel B*: tertiles of HDL-cholesterol/total cholesterol ratio with decreasing risk gradient. *Grey columns*: observed RRs. *Black columns*: RRs computed under the hypothesis of no interaction between paired risk factors. The lack of statistically significant differences between grey columns (observed RRs) and the adjoining black columns (computed RRs) excludes significant multiplicative interaction between the paired variables (Chi^2 (2DF) = 1.26, NS for IL-6, and Chi^2 (2DF) = 0.38, NS for HDL-cholesterol/total cholesterol ratio).

Table 3
Standardized relative risk of retinol and the strongest risk factors for CHD: The PRIME Study.

	Standardized RR	(95% CI)	*P*
[a]Retinol absolute (log)	0.71	(0.57–0.88)	0.002
Lipid-standardized retinol (log)	0.65	(0.52–0.81)	0.0001
[b]HDL-cholesterol/total cholesterol	0.53	(0.36–0.77)	0.001
Interleukin-6 (log)	1.56	(1.14–2.14)	0.006
Retinol absolute (log)	0.70	(0.53–0.93)	0.01
Smoking	1.35	(1.06–1.72)	0.02
Systolic blood pressure	1.30	(1.00–1.67)	0.05

Standardized RR denotes the RR observed with an increase of a variable by 1 SD of control levels before (upper panel) and after multivariate adjustment (lower panel). Variables, e.g. carotenoids, consumption indices for alcohol and fruit/vegetables, Body mass index, and total cholesterol failed to enter this regression model with statistical significance ($P > 0.05$).

[a] Univariate conditional logistic regression.

[b] Multiple conditional logistic regression adjusting for the listed covariates of statistical significance.

cholesterol, triglycerides, Body mass index, hs-CRP, fibrinogen and alcohol intake (not shown) lacked significance in this model. Although HDL-cholesterol/total cholesterol ratio was the key explanatory variable, other analyses identified HDL-cholesterol as equally strong. None of the interaction terms between low retinol and the levels of carotenoids was significantly associated with CHD (not shown).

The combination of low retinol with tertiles of major risk factors, e.g. IL-6 (Fig. 2: Panel A) and the HDL-cholesterol/total cholesterol ratio; Panel B displayed independent, additive risk. Multiplicative interaction was excluded over the range of covariates. The strongest CHD risk was observed for low retinol and high IL-6 and with low HDL-cholesterol/total cholesterol ratio and HDL-cholesterol. Case numbers were inadequate for multiple risk factor testing.

4. Discussion

4.1. Carotenoids

In PRIME, as in the Physicians' Health Study [13], low carotenoid levels did not predict CHD. This contrasts with results in healthy middle-aged US smokers [14], and in Swiss with a 42% smoking prevalence [15]. Since smoking was strongly predictive in PRIME and was associated with low carotenoid levels, these results [14,15] may have been due to confounding by lifestyle factors, e.g. fruit/vegetable intake.

4.2. Vitamin A

In PRIME, low plasma retinol predicted CHD independently of carotenoids (Tables 2 and 3). This is plausible because vitamin A is only partly replaced by β-carotene (its major precursor) [1,2,16], since, in industrialized countries, conversion is poor and inconsistent [17]. Many studies have found no significant increase in plasma retinol associated with intake or plasma levels of carotenoids, and vitamin A-deficiency is not fully remediable by carotenoids in a herbivorous diet [16]. Thus the contribution of potential vitamin A-precursor [1,2] to vitamin A status may be overestimated in middle-aged westernised men.

There was a 9% significantly lower geometric mean in plasma retinol in cases than controls (Table 1), which was probably related to the risk associated with retinol levels <601 μg/l (quintile 1, Fig. 1). Previous studies may have failed to detect this because of adequate retinol levels [18–20]. Presumably, this was also the case in US Physicians whose retinol lacked any prediction of CHD [13]. A Dutch study in men and women of broad age-range, which included low retinol levels [21] probably lacked significance because of gender differences and the age-dependent rise in plasma retinol in males [1,22].

Research on vitamin A initially concentrated on severe A-avitaminosis in children in under-developed countries which may be prevented at plasma retinol levels ≥284 μg/l [1,2], whereas in adult men A-hypovitaminosis with anaemia, cytological and functional abnormalities, can occur at ≤400 μg/l [1,12]. In PRIME subjects the plasma retinol generally lay in the 500–1000 μg/l range, previously considered as safe [1]. The association of CHD with the lower end of this range (<600 μg/l) suggests that these men could benefit from levels of 750–850 μg/l (avoiding A-hypervitaminosis A [1,2] at >940 μg/l).

Factors affecting retinol levels are complex, but the intake of preformed retinol [1] may be crucial since, under steady-state conditions, it has a weak, albeit significant, positive association with plasma retinol in healthy adult males, as in NHANES III [22]. Thus, our results reinforce the current Recommended Daily Intake (or 'Allowance') of at least 0.7–1 mg (safe range ≥3 mg in adult males) [1,2]. Vitamin A status would best be improved from preformed vitamin A, preferably from natural sources [1], which are unlikely to cause overdose.

Strengths of the PRIME Study include its prospective design and the assessment of multiple risk factors. A weakness is the limited number of events and the modest number of controls, so confirmation of our findings is crucial. Of the numerous confounders of plasma retinol [1,2], the most obvious are:

- *Lipids*: the weak positive association of cholesterol and triglycerides with absolute levels of retinol disappeared after lipid-standardization [11] (Table 2). Secondly, retinol and lipids behave inversely regarding CHD (Table 3). Thirdly, regardless of the positive correlation of retinol with HDL-cholesterol/total cholesterol ratio (Table 2), their combination results in an independent additive risk (Table 3; Fig. 2: Panel B) which is almost identical to the combination of retinol with HDL-cholesterol (not shown).
- *Alcohol*: the weak positive association between retinol and alcohol intake in controls (Table 2) and cases may be unimportant since globally – in contrast to France (data not shown) – alcohol consumption in cases and controls did not differ significantly (Table 1). Similarly, alcohol intake was not significant in multivariate analysis (Table 3).
- *Inflammation*: plasma retinol correlated inversely with fibrinogen and hs-CRP ($P < 0.01$) but not significantly ($P > 0.05$) with IL-6, which displayed the closest association with CHD [8] (Tables 1–3). Furthermore, the combinations of low retinol with rising IL-6 increased the CHD risk independently and additively (Fig. 2: Panel A).
- *Ageing*: any increase in plasma retinol in the narrow age range of 50–59 years cannot be substantial in PRIME males whose plasma retinol was not significantly correlated with age (Table 2). Nevertheless, all controls were carefully matched for age.
- *Season*: any effect was excluded by matching.
- *Medication*: all subjects with CHD at baseline were excluded from analyses. Some apparently healthy subjects took hypotensives (HR = 1.83; CI 1.10–3.06) but this did not correlate significantly with absolute and lipid-standardized retinol in cases or controls. This also held for hypolipidaemics.
- *Other*: in controls, absolute levels of retinol were unrelated to cigarette smoking, diabetes, and Body mass index (Table 2). Absolute and lipid-standardized retinol also varied independently of the number of years of schooling (a proxy for social class).

We believe these are unlikely to contribute to the increased CHD risk we observed at low plasma retinol levels.

5. Conclusion

These novel results suggest that plasma retinol levels of <601 μg/l in a fifth of middle-aged European men place them at an approximately threefold RR of developing CHD. This acts independently, but additively to HDL-cholesterol and IL-6 and possibly to other risk factors, e.g. smoking and systolic blood pressure. Our findings await replication, particularly as to whether increasing the intake of preformed vitamin A can correct low vitamin A levels and reduce CHD risk.

Conflict of interest

None.

Acknowledgements

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Appendix A. The PRIME Study Group

The PRIME Study is organized under an agreement between INSERM and the Merck, Sharpe and Dohme-Chibret Laboratory, with the following participating Laboratories:

- The Strasbourg MONICA Project, Laboratoire d'Epidemiologie et de Sante Publique, EA 3430, Strasbourg F-67085, France; and Universite Louis Pasteur, Strasbourg, F-67085, France (D. Arveiler, B. Haas).
- The Toulouse MONICA Project, INSERM, U558; and Département d'Epidemiologie, Universite Paul Sabatier - Toulouse Purpan, Toulouse, France (J. Ferrières, J.B. Ruidavets).
- The Lille MONICA Project, INSERM, U744, Lille, France; and Institut Pasteur de Lille, Lille, France; Université de Lille 2, Lille, France (P. Amouyel, M. Montaye).
- The UKCRN Centre of Excellence for Public Health (NI), Queen's University of Belfast, UK (A. Evans, J. Yarnell, F. Kee). The Department of Atherosclerosis, INSERM, U545, Lille; Institut Pasteur de Lille, Lille; Université de Lille 2, Lille, France (G. Luc, J.M. Bard).
- The Laboratory of Haematology, INSERM, U626, Marseille, Hôpital La Timone, Marseille, France (I. Juhan-Vague, P. Morange).
- The Laboratory of Endocrinology, INSERM, U563, Toulouse, France (B. Perret).
- The Vitamin Research Unit, The University of Bern, Bern, Switzerland (K.F. Gey).
- The Nutrition and Metabolism Group, Centre for Clinical and Population Sciences, Queen's University Belfast, Northern Ireland (J. Woodside, I. Young).
- The DNA Bank, INSERM, U525, Paris, France (F. Cambien).
- The Coordinating Centre, INSERM, Unit 909, Villejuif, F-94807, France; and Université de Paris V, Paris Cardiovascular Research Centre (PAARC), Paris, F-75015, France (P. Ducimetiere, A. Bingham).

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