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Correlations between Cholesterol, Vitamin E, and Vitamin K_1 in Serum: Paradoxical Relationships to Established Epidemiological Risk Factors for Cardiovascular Disease, *Bill E. Cham,[1*] Jeffery L. Smith,[2] and David M. Colquhoun*[3] ([1] The Curacel Institute of Medical Research, 14/1645 Ipswich Road, Rocklea, Qld 4016, Australia; [2] Lipid Metabolism Laboratory, Department of Surgery, The University of Queensland, Royal Brisbane Hospital, Herston, Qld 4029, Australia; [3] Wesley Medical Centre, Auchenflower, Brisbane, Qld 4060, Australia; * author for correspondence: fax 61-7-3274 4453)

The prime role of cholesterol in the pathogenesis of atherosclerosis is almost universally accepted. Vitamins E and K_1, two fat-soluble vitamins associated with lipoproteins, appear to have antiatheroma properties. Paradoxically, there are good linear correlations of vitamins E and K_1 with plasma cholesterol concentrations. From an epidemiologist point of view, both vitamins K_1 and E could be regarded as being atherogenic. From the point of view of a biochemist, these vitamins may be regarded as antiatherogenic.

Epidemiological studies demonstrate an exponential relationship between increased plasma cholesterol, specifically LDL-cholesterol, and coronary heart disease because of atherosclerosis. Mutations of both the LDL receptor and LDL may be responsible for the loss of recognition between the LDL receptor and the LDL particle, leading to high plasma cholesterol concentrations. Macrophages take up lipoproteins via two receptor-mediated processes, the LDL receptor and a scavenger receptor capable of binding and internalizing various "modified" lipoproteins. The scavenger receptor recognizes apoprotein B-containing lipoproteins that have been modified generally with an increased negative charge. This change may be experimentally induced by acetylation or by reaction with malondialdehyde *(1)*. In vivo peroxidative modifications of apoprotein B produce a series of changes of this apoprotein, which render it recognizable and ingestible by the scavenger receptor *(2–5)*. This receptor is essentially unregulated, in contrast to the classical LDL receptor, and consequently, pronounced accumulation of cholesteryl ester in macrophages ensues, producing foam cell formation, the hallmark of atheroma *(5)*.

Vitamin E inhibits lipid peroxidation and prevents formation of malondialdehyde. Independent studies *(6, 7)* have shown that modification of LDL by cells that produces increased susceptibility to oxidation is inhibited by antioxidants such as vitamin E. Consequently, it may be deduced that vitamin E has antiatheroma properties. Calcification occurs early in the development of atherosclerotic plaques. Calcium phosphate (hydroxyapatite) precipitates by a mechanism similar to active bone formation and is vitamin K-dependent *(8)*. γ-Carboxyglutamic acid (Gla)-proteins have been identified in calcified atherosclerotic plaques. The formation of Gla-proteins is dependent on vitamin K. These Gla-containing proteins have a very high affinity for hydroxyapatite. The only known function of these proteins is to bind calcium *(9–11)*. It has been suggested that Gla-proteins may be actively related to atherosclerotic calcification *(11, 12)*. Decarboxylation of Gla residues to glutamyl residues greatly diminishes the affinity of Gla-containing proteins for hydroxyapatite *(9–11)*. Paradoxically these Gla-proteins may inhibit precipitation and do not interfere with normal calcium homeostasis *(13)*. Thus, these proteins may have opposing roles of facilitator or inhibitor of calcification of plaques depending on other local factors. Atherosclerotic arteries contain only ~30% of the carboxylase activity formed in healthy arterial segments *(14)*. Vitamin K, in addition, has antioxidant properties *(15)*. Thus vitamin K, like vitamin E, appears to have antiatheroma properties, with vitamin K being a promoter of dystropic calcification in certain circumstances.

It has been reported previously that concentrations of vitamin E in serum vary depending on the amount of lipid *(16)* and apoproteins (Cham et al., submitted for publication). We now present further evidence that there are significant correlations between wide concentration

Table 1. The concentrations of cholesterol, vitamin E, and vitamin K_1 in the sera of neonates and fasting adult normocholesterolemic and hypercholesterolemic subjects.[a]

	Cholesterol, mmol/L	Vitamin E, mg/L	Vitamin K_1, μg/L
Neonates	1.54 ± 0.34	2.51 ± 1.01	83 ± 120
(n = 20)	(0.8–2.2)	(0.3–5.3)	(24–103)
Normocholesterolemic subjects	4.76 ± 0.56	7.91 ± 1.49	343 ± 155
(n = 21)	(3.7–5.5)	(5.5–11.7)	(49–590)
Hypercholesterolemic subjects	6.72 ± 0.86	11.93 ± 2.97	564 ± 195
(n = 16)	(5.7–9.2)	(6.0–17.7)	(204–910)

[a] The results are expressed as mean ± SD; the concentration ranges are shown in parentheses. Serum cholesterol was measured by enzymatic methods *(21)*, whereas vitamin E and vitamin K_1 concentrations were assayed by established simultaneous liquid chromatography procedures *(22)*.

ranges of cholesterol, vitamin E, and vitamin K_1 in serum, which pose some interesting questions. Concentrations of these components were measured in serum from neonates and fasting normolipidemic and hyperlipidemic adults. The procedures used for these human studies were in accord with the Helsinki Declaration of 1975, as revised in 1993. Serum specimens contained a wide range of cholesterol (0.8–9.2 mmol/L), vitamin E (0.3–17.7 mg/L), and vitamin K_1 (24–910 μg/L) concentrations (Table 1). The correlation coefficients derived from the intraclass correlations pooled over the three groups were as follows: cholesterol vs vitamin E ($r = 0.922$, $P \ll 0.001$, n = 57), cholesterol vs vitamin K_1 ($r = 0.729$, $P \ll 0.001$, n = 56), and vitamin E vs vitamin K_1 ($r = 0.654$, $P \ll 0.001$, n = 56). The three groups of subjects had significantly different serum concentrations of cholesterol and vitamins. The significant differences disappeared when vitamin E and vitamin K_1 concentrations were related to total cholesterol concentrations in serum. The partial correlations between vitamin E and cholesterol in serum expressed as the ratio vitamin E(μmol/L)/total cholesterol(mmol/L) ± SD were 3.8 ± 1.4 for neonates, 3.9 ± 0.6 for normocholesterolemic adults, and 4.1 ± 0.9 for hypercholesterolemic patients. The partial correlations between vitamin K_1 and cholesterol in serum expressed as the ratio of vitamin K_1(nmol/L)/total cholesterol(mmol/L) ± SD were 0.13 ± 0.18 for neonates, 0.16 ± 0.08 for normolipidemic adults, and 0.19 ± 0.06 for hypercholesterolemic patients. We also present data of a male patient with severe hyperlipidemia who was treated for this abnormality by diet modification. The extent of the diet-induced cholesterol changes was linearly related to the extent of both vitamins E and K_1 changes (Fig. 1). The correlation coefficients derived from the Marquardt-Levenberg algorithm using SigmaPlot 4.0 curve fitter were as follows: cholesterol vs vitamin E ($r = 0.98$, $P \ll 0.001$), cholesterol vs vitamin K_1 ($r = 0.98$, $P \ll 0.001$), and vitamin E vs vitamin K_1 ($r = 0.98$, $P \ll 0.001$).

It would appear from the above data that the variability of the absolute serum vitamin E and Vitamin K_1 concentrations may depend on that of the concurrent serum cholesterol concentrations. Accordingly, the assessment of vitamin E and vitamin K_1 status should always take into account the lipid concentrations, as has been proposed previously for vitamin E *(16–20)*. Therefore, statements about the effect on cardiovascular disease of these two vitamins should be done with lipid adjusted values. The current observations highlight some very interesting propositions. On the one hand, because of the observed correlations of vitamins E and K_1 with serum cholesterol and because serum cholesterol is related to the incidence of atherosclerosis, from an epidemiologist point of view, both vitamins E and K_1 could be regarded as being atherogenic. On the other hand, because of the known biological properties of vitamins E and K as described above, from the point of view of a biochemist, these vitamins may be regarded as being antiatherogenic. From a clinical point of view, these data are confusing because, by reducing serum cholesterol to obtain a more desirable

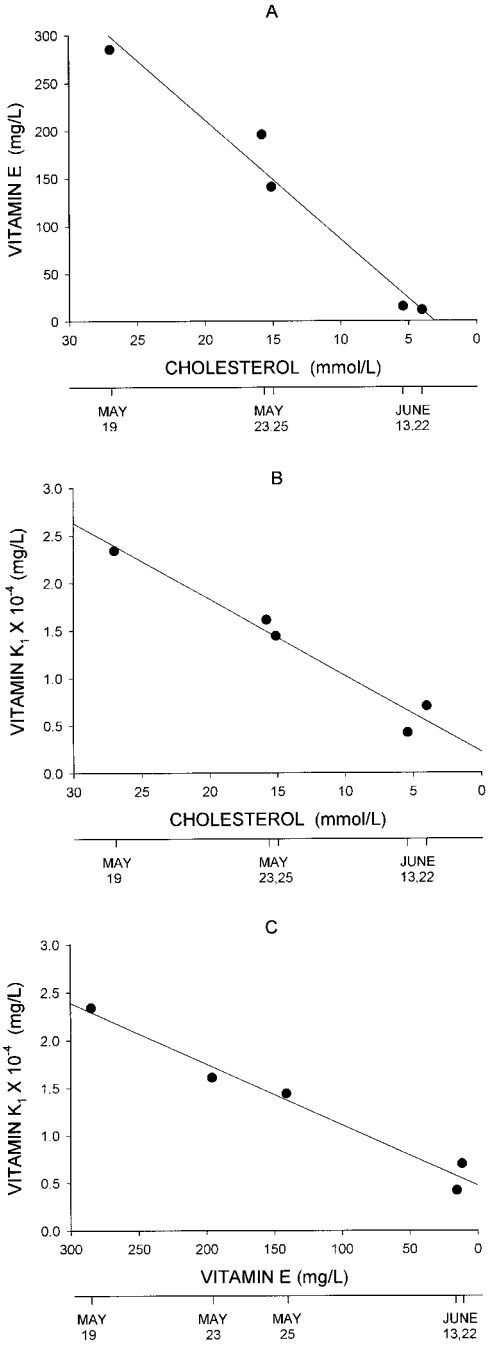

Fig. 1. Correlations of cholesterol and vitamin E (*A*), cholesterol and vitamin K_1 (*B*), and vitamin E and vitamin K_1 (*C*) concentrations in sera of an initially severe hypercholesterolemic male patient, according to the dietary guidelines of the National Heart Foundation of Australia.

The patient was diagnosed as a non-insulin-dependent diabetic and had no other known medical condition. Before dietary modification, the patient was on an average daily energy diet of 23567 kJ consisting of 15% protein, 41% fat, and 44% carbohydrate. The composition of the fatty acids was 42% monounsaturated, 21% polyunsaturated, and 37% saturated. Cholesterol consumption was 520 mg/day. The patient was prescribed a low fat-diabetic-weight reduction diet, which consisted of daily intake of 6000 kJ, of which 27% was derived from protein, 17% from fat, and 56% from carbohydrate. The composition of the fatty acids was 38% monounsaturated, 31% polyunsaturated, and 31% saturated. Cholesterol consumption was 116 mg/day. Within 1 month of the change of the diet, the serum cholesterol concentration was reduced from 27 mmol/L to 4 mmol/L.

concentration of cholesterol, a concomitant reduction occurs in vitamins E and K, two components that are considered antiatherogenic. What are the clinical implications of these observations?

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Discrimination between Celiac and Other Gastrointestinal Disorders in Childhood by Rapid Human Lymphocyte Antigen Typing, *Lucia Sacchetti,[1] Giuseppe Calcagno,[1] Anna Ferrajolo,[1] Claudia Sarrantonio,[1] Riccardo Troncone,[2] Maria Micillo,[2] Salvatore Auricchio,[2] and Francesco Salvatore[1*]* ([1] Dipartimento di Biochimica e Biotecnologie Mediche, Facoltà di Medicina e Chirurgia, and CEINGE-Biotecnologie Avanzate, Università "Federico II", via S. Pansini 5, 80131 Napoli, Italy; [2] Dipartimento di Pediatria, Facoltà di Medicina e Chirurgia, Università "Federico II", via S. Pansini 5, 80131 Napoli, Italy; * author for correspondence: fax 39-81-7463650, e-mail salvator @unina.it)

Celiac disease (CD) is a genetically complex, multifactorial immune-mediated disease *(1)*. The intestinal damage that characterizes the disorder is induced by dietary gluten ingestion in susceptible individuals *(1)*. A specific HLA *DQA1*0501/DQB1*0201* heterodimer, or in a few cases, the HLA *DRB1*04* alleles, is associated with the disease *(2)*. In fact, when presented by these HLA molecules, gluten-derived peptides cause T-cell activation in the intestinal mucosa, which is followed by cytokine production and mucosal intestinal damage *(1)*.

The clinical manifestations of CD are very variable, and most of the symptoms are also present in other clinical conditions. Diagnosis of CD includes the presence of circulating gliadin and endomysium antibodies (EMAs) *(3)* and is traditionally confirmed by intestinal biopsy, according to European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) criteria *(4)*. The genetic diagnostic approach may be very useful in CD patients with IgA deficiency, a condition where the serological tools are less useful; in familial screening; or in cases of latent forms of CD. Regarding the genetic approach, despite numerous reports of HLA class II associations to CD in diverse countries *(5–9)*, there has been no systematic study to establish the role of HLA typing in the diagnosis of CD, particularly in discriminating CD from other confounding diseases.

The aim of this study was to verify, using a PCR-based method that we recently established *(10)*, the prevalence of the HLA heterodimer and of the HLA *DRB1*04* alleles in healthy subjects, in CD-affected children, and in other age-matched subjects affected by confounding disease from South Italy. In a subgroup of CD patients in an active stage of disease, we also compared the diagnostic characteristics of genetic patterns and the presence of the EMAs and gliadin antibodies.

We examined two groups of patients from the Center for the Study of Gastrointestinal Disorders of the Department of Pediatrics of our University. One group (n = 74), affected by CD diagnosed according to the ESPGAN criteria *(4)*, was examined retrospectively. The other group consisted of 80 patients (mean age, 6 years) at their first clinical examination whose history of clinical symptoms and/or results from laboratory tests were suggestive of CD. All 80 patients underwent jejunal biopsy. Celiac disease was diagnosed in 48 of 80 patients on the basis of