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Dietary Vitamin A Intake and Risk for Hip Fracture

To the Editor: I read with interest Melhus and colleagues’ article on the association between vitamin A and bone mineral density and fractures (1). The authors might, however, further discuss the limitations of their study. The bone mineral density part of the study was cross-sectional and included 175 women who were 28 to 74 years of age. Univariate analysis did not show any significant association between bone mineral density and retinol intake (P > 0.2). In multivariate analysis, however, the authors found a significant association without clarifying which additional covariates made the effect of retinol significant. For example, it is well known that intake of retinol and intake of vitamin D are highly correlated. The authors’ Table 3 shows that the 95th percentile for retinol intake is 1.58 mg/d; thus, we can calculate (the authors do not show this) that approximately 11% of the participants must have been in the group with retinol intake greater than 1.5 mg/d. Only this small group was significantly different (and, notably, only in multivariate analysis) from the group with retinol intake less than 0.5 mg/d (the authors’ Table 5). The main finding in the fracture study (a case–control study) is also based on the group with retinol intake greater than 1.5 mg/d, which must have consisted of fewer than 10 case-patients. It is not clear whether the authors adjusted for vitamin D intake.

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Reference

In response: Dr. Sigurdsson would like clarification of the covariates that made the effect of retinol significant in the multivariate analysis in the bone mineral density study. He would also like to see the results after adjustment for vitamin D intake.

We tried to include the same set of covariates in both the hip fracture and bone mineral density studies (established osteoporosis risk factors). No major confounding was observed in the hip fracture study, but in the linear regression analysis of the bone density study, inclusion of possible confounders did change our estimates. Energy intake was the covariate with the most pronounced effect on the β estimates in the latter analysis. The bone density study included a more heterogeneous sample of women with regard to age and menopausal status. When we restricted the analysis of retinol intake to a dichotomous variable (> or < 1.5 mg/d), no major differences were seen between univariate and multivariate analysis (Table 5 of our article).

Dr. Sigurdsson argues that because the correlation between the intake of vitamin A and intake of vitamin D is usually 0.8 to 0.9, our result may be explained by a high vitamin D intake. The correlation coefficient between these two nutrients in the bone mineral density study was 0.4. In the last sentences of the Results section, we note that adjustment for intake of several other nutrients does not significantly change the association of retinol intake and bone mineral density. In fact, the β-coefficients shown do include adjustment for vitamin D intake. Unfortunately, we did not include this information in the final version of the article.

We thank Dr. Sigurdsson for drawing our attention to this.

With regard to the fracture study, Dr. Sigurdsson states that the main finding is based on the group with an intake greater than 1.5 mg/d, which must have contained fewer than 10 case-patients. Table 3 clearly indicates that this calculation is incorrect. The true numbers are 33 case-patients and 66 controls.

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Vulnerable Plaque

To the Editor: Kullo and colleagues have presented an excellent review of the cellular and molecular aspects of a vulnerable plaque (1). Their Table 1 mentions the cellular composition of rupture-prone plaques and lists macrophages, T cells, and mast cells. However, although their discussion involves the role of macrophages, T cells, and smooth-muscle cells, little attention was paid to the potential role of mast cells in plaque instability or rupture.

Kaartinen and colleagues (2) demonstrated very clearly that mast cells localize to areas of erosion or rupture sites of atheromas, and these cells expressed tumor necrosis factor-α. Mast cells have also been shown to aid in the formation of foam cells by assisting smooth-muscle uptake of low-density lipoprotein remnants (3). More recently, Johnson and coworkers (4) investigated the role of mast-cell proteases in activating matrix-degrading metalloproteinases (MMPs) in human carotid endarterectomy specimens. By stimulating mast cells to degranulate in vitro by adding the compound 48/80 to atherectomy samples, these investigators showed increased MMP activity, a process inhibited by the tryptase inhibitor antipain and the chymase inhibitor chymostatin (4). Using dual immunocytochemistry, these investigators also showed co-localization of MMP-1 and MMP-3 with mast cells in the shoulder region of plaques, where the maximal numbers of degranulated mast cells also occurred.

As we recently summarized, mast cells are fascinating cells...
To the Editor: In their excellent review, Kullo and colleagues did not mention colchicine, a potent agent that precisely and specifically inhibits the very protagonists of plaque formation—desaturation and rupture. Colchicine binds to β-tubulin and inhibits the proliferation of microtubules and microtubule-dependant processes during cell activation. This affects not only lymphocyte proliferation and the migration, diapedesis, and phagocytic activity of granulocytes and macrophages but also the synthesis, intracellular transport, and secretion of cytokines (2). Colchicine decreases tumor necrosis factor and interleukin-2 secretion, downregulates tumor necrosis factor receptors on macrophages, and deactivates these receptors’ secretion of proteases and other mediators. All of this can destabilize the plaque, lead to rupture, and activate thromboocyte aggregation and intramural coagulation. Moreover, colchicine decreases the adhesiveness of neutrophils to endothelial cells by changing the distribution of E-selectin molecules on their surface, which are activated by cytokines (3).

Colchicine is a rapidly effective, cheap, well-known anti-inflammatory and immunosuppressive drug that has low toxicity and does not interfere with antiangiinal, antiinflammatory, or anticoagulant medications. It could have a place of primary importance in the emergency treatment of unstable angina pectoris and preinfarction syndromes.

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In response: Krishnaswamy and colleagues outline several mechanisms by which mast cells might contribute to plaque inflammation and rupture. Further work is needed to establish a direct role for mast cells in plaque instability. The hypothesis that plaque behavior could be favorably altered by preventing mast-cell degeneration or by inhibiting mast-cell proteases is intriguing but as yet unproven. Drs. Doherty and McMillen point out the several proatherogenic properties of endothelin-1. The work by Zeiher and coworkers (1) suggests that endothelin-1 is involved in the pathogenesis of acute coronary syndromes. However, evidence for endothelin-1 as an important determinant of plaque vulnerability and rupture is yet to come. With the introduction of endothelin-1 antagonists, an opportunity exists for further exploration of the role of endothelin-1 in the behavior of atherosclerotic plaques. If endothelin-1 antagonists do reduce coronary events, a central role for endothelin in promoting plaque instability could then be postulated.

Dr. de’ Clari proposes that colchicine may have a stabilizing effect on atherosclerotic plaques. Colchicine binds tubulin and disrupts the microtubular system in granulocytes and other monocyte/macrophages, leading to a decrease in chemotaxis, metabolic and phagocytic activity, and resulting anti-inflammatory effects. Because of these properties, the drug has been tested for antiatherogenic effects in animal models. The results have been inconsistent. In cholesterol-fed rabbits, colchicine decreased atheroma formation (2), but it had no effect in a swine model of
aortic atherosclerosis (3). Although effects on macrophage cyto-
kine production and metalloproteinase activation (4) may be
beneficial, the inhibition of fibrosis by colchicine (5) could be
detrimental to plaque stability. At present, the therapeutic po-
tential of colchicine, if any, in atherosclerosis is unclear; further
studies are needed.

The issues raised by the respondents highlight the need for
animal models of plaque rupture in which these hypotheses could be
tested.

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Decrease in Endothelin-1 Plasma Levels during the
Menstrual Cycle and after Ethinylestradiol
Treatment

To the Editor: The endothelium regulates vascular tone and
growth by releasing factors such as endothelin-1 and nitric oxide
(1). Impaired endothelial function and elevated levels of endo-
thelin-1 contribute substantially to cardiovascular disorders (2–4).
Oral contraceptives containing ethinylestradiol are associated
with an increased cardiovascular risk (5). It is not known whether
this risk is caused by changes in endothelium-derived factors.

We investigated whether 7-day therapy with ethinylestradiol,
50 μg/d, causes changes in the potent vasoconstrictor endothe-
lin-1. Participants were 10 healthy premenopausal women (mean
age, 32.4 years). Three fasting blood samples for the measurements
during early follicular development (days 2 through 4); sample 2, 7 days after sample 1; sample 3, cycle days 20 through 24. To correspond with the pretreatment cycle, the
blood samples during the treatment cycle were taken at cycle
days 2 through 4 (before treatment) and 7 days after the first
sample (after treatment). During the pretreatment cycle, endo-
thelin-1 levels decreased significantly (from 3.89 ng/L to 2.93
ng/L; P < 0.04) whereas estradiol levels increased (Figure). Ethinyl-
estradiol treatment led to a significant decrease in endothelin-1
levels compared with baseline levels during the treatment cycle
(from 2.94 ng/L to 2.26 ng/L; P < 0.03) and compared with all
measurements during the pretreatment cycle.

We conclude that for studies concerning endothelin-1 during
premenopause, investigators must consider that levels of this
peptide change during the menstrual cycle. Ethinylestradiol treat-
ment significantly decreases plasma endothelin-1 levels. This
finding indicates that this potent synthetic estrogen can profound-
ly affect an endothelium-released substance. This, in turn, might
influence the cardiovascular risk seen with oral contraceptive use.

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Correction: Cardiorespiratory Fitness, Impaired
Fasting Glucose, and Type 2 Diabetes

In an article on cardiorespiratory fitness and impaired fasting
glucose and type 2 diabetes (1), the last sentence of the second
full paragraph on page 93 should read “A decrease in fitness of
1 MET was associated with a 20% (CI, 10% to 26%) increase in
risk for impaired fasting glucose and a 28% (CI, 12% to 47%)
increase in risk for type 2 diabetes,” not an “increase in fitness of
1 MET…”

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