Commentaries

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Boning up on vitamin K

The nature and significance of the relationship between vitamin K status and bone health has been debated for some years. Vitamin K is required for the gamma carboxylation of glutamic acid residues in three bone proteins, namely osteocalcin, matrix Gla protein, and protein S. Although the roles of these proteins have not been clearly defined, there is evidence that osteocalcin, which is produced by cells of the osteocyte/osteoblast lineage and has three vitamin K dependent gamma carboxyglutamic acid (Gla) residues, may be involved in the mineralisation of bone matrix¹; in addition, it may function as a negative regulator of bone formation, deletion of the osteocalcin gene in mice resulting in increased bone mass.² Approximately 30% of synthesised osteocalcin is released into the circulation and serum levels of the protein are widely used as an indicator of bone formation.³ Vitamin K deficiency is associated with a reduction in circulating osteocalcin concentrations and a decrease in the gamma carboxylated fraction, the latter being associated with reduced binding of the protein to bone mineral.⁴ Despite this theoretical basis for adverse effects of vitamin K deficiency in the skeleton, however, direct evidence is lacking. Thus although low vitamin K levels and undercarboxylation of osteocalcin have been described in patients with osteoporosis,⁵⁻⁶ these findings may reflect poor nutritional status rather than a specific effect of vitamin K deficiency on bone. Similarly, there is currently no direct evidence that therapy with warfarin, which inhibits the secretion by osteoblasts of osteocalcin,⁷ increases the risk of osteoporosis, and although increased fracture risk has been reported in patients receiving oral anticoagulants, this finding has not been universal.

Malabsorption of fat soluble vitamins is a recognised complication of Crohn's disease and, in particular, an increased prevalence of vitamin D deficiency has been reported in many studies.⁸ In rare cases this results in osteomalacia but more importantly it contributes to the increased prevalence of osteoporosis associated with Crohn's disease as a consequence of secondary hyperparathyroidism and the resulting increase in bone turnover. In contrast, vitamin K status has been less well studied although in one study a high prevalence of deficiency of the vitamin was found in patients with ileal Crohn's disease.⁹ The study by Schoon *et al* in this issue of *Gut*, performed in a cohort of patients with small intestinal Crohn's disease, provides some evidence, albeit indirect, that there may be an inverse association between vitamin K status and bone mineral density of the spine (see page 473).¹⁰ Thus undercarboxylation of osteocalcin was higher and binding of circulating osteocalcin to hydroxyapatite lower in patients with Crohn's disease compared with controls, indicating reduced vitamin K status. Furthermore, serum levels of undercarboxylated osteocalcin were inversely related to bone mineral density in the spine, although this relationship was not observed for proximal femur or total body bone mineral density. Interestingly, although there was a significant correlation between serum levels of the

major circulating metabolite of vitamin D, 25-hydroxyvitamin D, and serum vitamin K, no correlation was observed between vitamin D status and bone mineral density at any of the sites assessed.

The results of this study would thus be consistent with a role for vitamin K deficiency in the pathogenesis of osteoporosis associated with Crohn's disease. Nevertheless, further studies are required in view of the relatively small sample size in this study (n=32) and lack of a statistically significant correlation between serum vitamin K levels and bone mineral density. In addition, the reduction in bone mineral density was relatively modest in this cohort, with mean z scores higher than -0.5 at all sites, and in this respect it would be of interest to investigate the relationship between vitamin K status and bone mass across a wider spectrum of bone disease. Failure to demonstrate a correlation between the serum "free" (undercarboxylated) osteocalcin and bone mineral density in the femoral neck and total body may reflect inadequate statistical power or alternatively might indicate differential effects on cancellous and cortical bone.

The pathogenesis of osteoporosis in patients with Crohn's disease is multifactorial, glucocorticoid therapy, hypogonadism, vitamin D deficiency, and malnutrition all being potential contributory factors.⁸ In the study of Schoon *et al*, patients were in remission at the time of assessment and were treated with no more than 5 mg of oral prednisolone daily. Nevertheless, bone mineral density values assessed at one point in time reflect multiple past and present influences and thus the effects of glucocorticoids and disease activity could not be excluded completely, as the authors claimed. The question of whether vitamin K supplementation prevents or reduces bone loss in patients with Crohn's disease (or in other populations) has not been established and this will be an important area for future research if an association between vitamin K status and bone mineral density is confirmed in subsequent studies.

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Bugs and the gut: breaking barriers to understanding

There has been a re-emergence of interest in the interplay between the faecal microbiota and its associated metabolisms and gastrointestinal function. This has recently been driven by the current interest in human and veterinary probiotics, and in the recent past by hypotheses which linked gut bacterial metabolism of ingested foodstuffs and colorectal and other cancers. However, what is less widely known is the extent of involvement of gastrointestinal bacteria in gut function, gut disease, and particularly diseases of other organs.

Some of the less generally well known aspects of this interplay relate to gut bacterial metabolites: for example, the production of volatile fatty acids exacerbating acidaemias such as methylmalonic acidaemia, and the associated role of antibiotics in patient management.¹ An area where there has been a resurgence of interest is the production of phytoestrogens from dietary precursors and their possible role in protection from hormone dependent breast cancer.^{2,3} The role of such metabolites in infertility in humans remains unexplored. There is even evidence that gut bacteria can induce or repress liver cytochrome P450 due to metabolism of dietary glucosinolates.⁴

There is a great deal of evidence to support the fact that what is considered normal gut structure and function is the end point of a complex set of interactions between the host and microbes colonising the gut.⁵ Such normal features include gastrointestinal motility, secretion and absorption, cell composition, mitotic activity, and villous length, etc. To this we can now add the fact that normal components of the gut microbiota can alter colonic barrier function by modifying colonic wall permeability (see page 503)⁶. This interesting observation raises a whole series of questions. The rationale behind the study is the hypothesis that increased intestinal permeability is a key factor in the pathogenesis of idiopathic bowel disease and the proposal that permeability may be affected by gut bacteria. What was found in the rat model used was that none of the test organisms increased permeability to a large molecular probe (dextran) but some altered permeability to a small molecular probe (mannitol). Interestingly, this modification in permeability was not uniform, with reduction, increase, and no significant alteration being detected, dependent on the test organism used. What would be interesting to know is the extent to which such effects are a

feature of the bacterial strain used or a feature of the bacterial species represented. For example, do most commensal *Escherichia coli* increase permeability or only a few strains? This leads to the deeper question of whether or not patients with idiopathic bowel inflammation are more frequently colonised with bacteria that increase gut permeability and/or have a gut microbiota where the net effect is to increase permeability. Both questions can now be answered. I would be particularly interested to see the effect of crude gut "flora" from, for example, patients with Crohn's disease, on gut wall permeability in small and large bowel in the "rat-human flora" model.

Similarly, it would be of interest to know whether or not the decrease in permeability induced by the isolate of *Lactobacillus brevis* is strain specific or a general feature in the species or genus. This may be of particular relevance in the probiotic field. A minor disappointment with the current paper is that the opportunity was not taken to see if coadministration of the *L. brevis* strain with the *E. coli* strain would reverse or prevent the increased permeability associated with *E. coli*.

There are many other questions which come to mind such as: could an ability to increase permeability be a virulence factor which facilitates translocation of a bacterium or its toxin—for example, verotoxin across the gut wall? Are changes in permeability due to specific factors produced by these bacteria or are they a result of metabolic byproducts? What is the range of degrees of altered permeability induced by different bacteria?

I have little doubt that this paper will stimulate research in these areas and that the results of such studies will offer increasing insights into the complex interplay between the host and its microbiota, and a better understanding of what is normal and what is abnormal.

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The changing scope of colorectal cancer

The prognosis in colorectal cancer depends on the stage at which the disease is diagnosed. Patients with advanced disease usually die of cancer, but when large intestinal tumours are found at an early asymptomatic phase a cure can be anticipated. Furthermore, the premalignant

lesions—adenomatous polyps—grow in the colon for years and perhaps decades before malignant conversion occurs, providing an opportunity for their removal, interrupting the natural history of these neoplasms. It has long been recognised that preventive strategies would be appropriate for this disease, and an extensive literature can be found on the subject

Two general approaches to detecting asymptomatic early staged colorectal cancers have been studied in depth. The first is the use of faecal occult blood tests as colorectal neoplasms

add blood to the stool that can be detected prior to the development of symptoms. However, this approach is relatively weak due to deficiencies in both sensitivity and specificity. One can expect a reduction of colorectal cancer mortality of <20% if the test is performed every other year.¹⁻³ False positive tests greatly outnumber true positives, all of which require a full investigation. The test is easy and non-invasive, but compliance is poor. This approach is often used but enthusiasm for this should be limited. The second approach is to perform endoscopic screening on asymptomatic individuals and remove the premalignant lesions. Although all of the evidence for efficacy of the latter approach is either retrospective or uncontrolled, the reduction in cancer mortality might be as high as 70–80%, and it may be sufficient to perform these examinations only once every 5–10 years to achieve this outcome.^{4,5}

Flexible sigmoidoscopy will readily detect neoplasms as far as the splenic flexure in unsedated patients. The traditional autopsy based literature from the mid-20th century indicated that as many as 75% of all colorectal neoplasms occurred in the distal colon. With this distribution, sigmoidoscopy would be an excellent (though imperfect) means of finding early colorectal cancers. However, if the distribution of colorectal lesions should shift, the impact of this procedure would change accordingly. It has been reported from North America and Europe that over the past several decades the location of colorectal neoplasms is shifting from the left to the right side of the colon. McCallion *et al* have communicated in this issue of *Gut* that when the distribution of colorectal cancers in Northern Ireland reported from 1990–97 was compared with those reported from 1976–78, a significant proximal shift had occurred; whereas only 23.5% of cancers were found proximal to the splenic flexure in the early time period, this number grew to 36.7% in the 1990s (see page 522).⁶ Is this really the case, and if so, what accounts for it?

Firstly, did this apparent proximal shift really occur or was it an artefact of data collection? It is possible that previously under counted proximal cancers are more accurately diagnosed by the use of current technology, including colonoscopy and imaging techniques. One would need to know how often patients died without an accurate diagnosis of a proximal colon cancer in the 1970s, and this may be difficult or impossible to solve. It is probably the case that there has been a fall in the number of deaths in the Northern Ireland registries that were attributed to abdominal cancer of unknown source, and the assumption is that proximal rather than distal cancers would have been more likely undiagnosed. It is perhaps worth noting that the two databases used by McCallion *et al* had substantial differences in ascertainment: the Northern Ireland Cancer Registry (NICR) reported 922.3 colorectal cancers/year whereas the Northern Ireland CRC registry reported only 644.1/year, as pointed out by the authors. The authors have rejected a significant impact of these differences, but in the NICR database, nearly 20% of colorectal cancers were of an unknown site.

Secondly, if this pattern shift is true, what is the mechanism for this change? A growth in public awareness of symptoms and prompt diagnosis of symptomatic cancers may lead to an improvement in mortality, but not in cancer incidence, which would remain unchanged. If a substantial proportion of the population had been subjected to screening sigmoidoscopy with the attendant removal of adenomas, this might lead to a substantial reduction in cancer incidence, especially in the distal colon and rectum. The authors do not present data on the frequency of flexible sigmoidoscopy for colon cancer screening during the two periods but it is possible that

there had been an increased use of flexible sigmoidoscopy (for screening or symptoms) during the 1990–97 period compared with the 1976–78 period. Such has been the case in the USA. If the later study group consisted of a more elderly population, it is expected that there would be a greater proportion of right sided colon cancers.⁷ Finally, perhaps another factor has intervened to alter the distribution of cancers within the colon. It has been demonstrated that aspirin and non-steroidal anti-inflammatory users experience a reduction in cancer mortality in the range of 40%, even for relatively occasional users.^{8,9} If additional factors such as increased caloric intake raised the general risk for cancer in the population while aspirin and related agents reduced the risk in the distal colon, there would appear to be a left to right shift at the population level. This remains a conjecture at present but it is likely that the factors responsible for the pathogenesis of proximal cancers may be quite different from those that lead to distal cancers.

Finally, what is the clinical impact of a true shift in colorectal neoplasms towards the proximal colon? Recent publications from North America explore the impact of screening with total colonoscopy.^{10,11} A substantial number of patients have important lesions above the reach of the sigmoidoscope without distal “signal” lesions. Patients in the USA have access to this information through the news media and Internet, and increasingly are insisting on a complete colonoscopic examination, particularly if they perceive themselves to be at an increased risk for cancer. Considerable investments of resources are at stake in this debate. Perhaps we should accept that only a full examination of the colon will suffice for purposes of screening. This would add pressure to more fully develop highly accurate non-invasive imaging techniques to screen the entire colon, such as virtual colonoscopy. Eventually genetic testing of stools might be sufficiently sensitive to act as a primary screening test.¹² We have come a long way since the first stool was tested with guaiac reagents. We need to adapt to the changing nature of the disease that might be occurring as we design optimal prevention programmes.

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