normal karyotype. Several studies have concluded that the morphologic defect of hydrops rather than the chromosome abnormality may be associated with the positive screen for Down syndrome and the subsequent identification of fetal Turner syndrome (5,14). More recently, Saller et al. (15) investigated maternal serum analyte concentrations in euploid pregnancies and concluded that nonimmune hydrops is associated with increased hCG. It has been reported that two fetuses with nuchal thickening and a subsequent probable diagnosis of Noonan syndrome were associated with a positive Down syndrome screen related to decreased AFP and increased hCG (16). It is not known whether those pregnancies with an identifiable cystic hygroma would have subsequently developed hydrops. Laundon et al. (7) included all fluid collection in the category of hydrops, including hygroma and cysts, and found increased free βhCG associated with Turner syndrome. Our data combined with that of Saller et al. (5), Knowles and Flett (4), and Laundon et al. (7) reveal that 30 of 34 fetuses with fluid collection (hydrops, hygroma, and cysts) were screen positive. In our sample of 19 fetuses, 4 (21%) had an increased Down syndrome risk and 1 (5%) had an increased trisomy 18 risk without abnormalities on ultrasound. Overall, 74% (14 of 19) of our cases had a positive screen: 2 with a neural tube risk, 11 with a Down syndrome risk, and 1 with a trisomy 18 risk. Recently, inhibin A concentrations were moderately decreased in cases of Turner syndrome without hydrops but markedly increased in cases of Turner syndrome with hydrops (17).

As has been reported previously, the maternal serum AFP and uE₃ concentrations were slightly reduced in pregnancies affected with Turner syndrome. In addition, hCG concentrations were increased in fetal Turner syndrome but more so in hydropic pregnancies. Based on these findings, some cases of Turner syndrome may be identified prenatally as a result of an increased risk for Down syndrome when these markers are used. Thus, women with an increased risk of Down syndrome based on multiple-marker screening should be counseled that Turner syndrome may be a possibility, even in the absence of fetal hydrops. The ascertainment of two fetuses with Turner syndrome as the result of increased trisomy 18 risk suggests that Turner syndrome may be given as a possible explanation for an increased trisomy 18 risk.

References

What Happens to Vitamin K₁ in Serum after Bone Fracture? Bill E. Cham'1 Jeffrey L. Smith,² and David M. Calquhoun³ (1 The Curacel Institute of Medical Research, 14/1645 Ipswich Rd., Rocklea Queensland 4106, Australia; 2 Lipid Metabolism Laboratory, Department of Surgery, University of Queensland, Royal Brisbane Hospital, Herston Queensland 4029, Australia; 3 Wesley Medical Centre, Auchenflower Brisbane Queensland 4060, Australia; * author for correspondence: fax 61-7-3274-4453)

We wished to investigate the mechanism of decreased serum vitamin K₁ after bone fractures. Vitamin K₁ plays a role in bone formation because it is required as a cofactor for the transformation of glutamic acid (Glu) residues on proteins to γ-carboxyglutamic acid (Gla) residues. The double carboxy group on Gla residues has high affinity for the binding of calcium. Bone formation involves vitamin K-dependent small peptide osteocalcin (bone-Gla-protein) that is secreted by osteoblasts.

Serum concentrations of vitamin K₁ reflect in part the capacity of the serum to carry the vitamin. Vitamin K₁ is a lipid, and little is known about the binding of this vitamin to proteins other than that it is transported in serum by the lipoproteins. It is not known whether there is a specific “vitamin K₁-binding protein” in tissues as has been suggested for vitamin E as a “tocopherol-binding protein” (1).

Deficiency of vitamin K will lead to defective γ-carboxylation of vitamin K-dependent proteins and will be
manifested by the failure of these proteins to function normally (2–8). Low serum concentrations of vitamin K₁ have been reported to occur in patients with traumatic bone fractures (4–6), although others have reported non-significant decreases in vitamin K₁ in the immediate 48 h after low-energy trauma hip fracture (9). These serum concentrations were considered pathological because they were significantly lower than values in age-matched control subjects. Recent studies, however, have shown that even very low absolute concentrations of vitamin K₁ in serum do not reflect the vitamin K status (3, 10, 11). The use of a relative measure of serum vitamin K₁, the ratio of vitamin K₁ to lipids (11) or to apolipoproteins (3, 12), has become essential in evaluating vitamin K₁ nutritional status as has been shown for vitamin E (11). Under routine conditions, the ratios of vitamin K₁ and vitamin E to other plasma lipoproteins components are relatively constant. Changes in the metabolism of lipoproteins such as are the case with hypercholesterolemia do not seem to affect such ratios (3, 11).

Stepwise linear regression methods have determined that serum concentrations of vitamin K₁ could best be predicted by using equations excluding lipids but containing only apolipoprotein A₁ and B concentrations: Vitamin K₁ (µg/L) = 369 × apolipoprotein B (g/L) × apolipoprotein A₁ (g/L). The correlation coefficient between the calculated values of vitamin K₁ using serum concentrations of apolipoproteins A₁ and B and the measured (HPLC) values of vitamin K₁ was 0.83 (3).

It has been suggested that the low serum concentrations of vitamin K₁ observed in patients with traumatic bone fractures was a consequence of sequestration of this vitamin from the circulation for use at the fracture site where it is required for the Gla transformation of special bone peptides (4).

LDL, which contains apolipoprotein B, is a negative acute phase reactant and is induced by inflammation (13, 14) such as with traumatic bone fractures. A decrease in synthesis and an increase in degradation of apolipoprotein B have been shown to occur. The reduction in apolipoprotein B concentration is reflected by a reduction in serum LDL concentrations as has been shown after acute myocardial infarction (13, 14). Consequently, because of the relationship of vitamin K₁ and apolipoprotein B (3), this may reflect the reduction in serum vitamin K₁ concentrations observed in patients soon after fracture. Thus it is possible that the lower concentration of vitamin K₁ in serum observed after bone fracture is attributable to a generalized lipoprotein carrier phenomenon (the negative acute phase response of LDL), which is an alternative explanation to the sequestration of vitamin K₁ from its carrier to bone fracture site.

To test whether the metabolism of vitamin K₁ in patients with bone fractures is altered independently of the lipoprotein carrier system, we measured vitamin K₁ (15) and apolipoproteins A₁ and B (3, 16) in sera from eight patients admitted for treatment of traumatic bone fractures in the pelvis. The sera were collected from all male patients (mean age, 45.0 years; range, 15–64 years) within 10 days (range, 1–9 days) of sustaining the fracture (4). The exclusion criteria were blood transfusions or surgical procedures before the period of sample collection and past and present illnesses related to bone metabolism. No subjects had been treated for osteoporosis, and none received medications before or during the study that might have affected calcium metabolism.

Serum samples were also taken from 12 healthy male subjects (mean age, 46.4 years; range, 19–55 years) for analyses of vitamin K₁, apolipoprotein A₁, and apolipoprotein B. There was no statistical difference (Student t-test) between the ages of controls and patients with fractures. Informed consent was obtained from all participants. The procedures used for these human studies were in accord with the Helsinki Declaration of 1975, as revised in 1996.

Serum vitamin K₁ concentrations from all subjects were also calculated by applying derived equations containing apolipoproteins A₁ and B concentrations (3).

The mean serum concentration of vitamin K₁ was significantly lower in the fracture patients than in the control subjects, but the concentrations of apolipoproteins A₁ and B were not significantly different in the two groups (Table 1).

When vitamin K₁ concentrations in serum were calculated by the equation: Vitamin K₁ (µg/L) = 369 × apolipoprotein B (g/L) × apolipoprotein A₁ (g/L) (3), the obtained mean value for the control subjects was 348 µg/L, similar to the value of 361 µg/L (Table 1). The ratio of corresponding individual measured/calculated values of vitamin K₁ in serum was 1.04 (SD = 0.04).

In contrast, the calculated mean concentration of vitamin K₁ from the bone fracture patients, 369 µg/L, was

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**Table 1. Concentrations of measured and calculated vitamin K₁ and measured apolipoproteins A₁ and B in the sera of healthy subjects and patients after bone fracture.**

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vitamin K₁ assayed, µg/L</td>
<td>Apolipoprotein B, g/L</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>361 ± 77ᵇ</td>
<td>0.73 ± 0.12</td>
</tr>
<tr>
<td>(n = 12)</td>
<td>(296–572)</td>
<td>(0.53–0.91)</td>
</tr>
<tr>
<td>Patients</td>
<td>221 ± 81ᵈ</td>
<td>0.93 ± 0.24</td>
</tr>
<tr>
<td>(n = 8)</td>
<td>(100–390)</td>
<td>(0.62–1.33)</td>
</tr>
</tbody>
</table>

ᵃ The results are expressed as mean ± SD; the concentration ranges are shown in parentheses. Vitamin K₁ concentrations were assayed by an established liquid chromatographic procedure (15). Apolipoproteins A₁ and B were measured by immunoassays (16). Vitamin K₁ was calculated by the formula: Vitamin K₁ (µg/L) = 369 × apolipoprotein B (g/L) × apolipoprotein A₁ (g/L) (3). The vitamin K₁ ratios of assayed and calculated values were from corresponding individual subjects. ᵇ–f Pairs differing significantly from each other at P < 0.01: ᵇ vs ᵈ, ⁴ vs ⁶; at P < 0.001: ᶜ vs ˡ. ³ ³
significantly different (P <0.01) from the measured mean concentration of 221 μg/L (Table 1). The ratio of corresponding individual measured/calculated values of vitamin K₁ in serum of bone fracture patients was 0.60 (SD = 0.13). This ratio was significantly different (P <0.001) from that of the control subjects (Table 1).

The current results confirm that the circulating serum vitamin K₁ concentrations are reduced shortly (within 10 days) after bone fracture (4–7). It is known that vitamin K₁ deficiency occurs in elderly subjects (6). However, in our study, because all the subjects were males and their ages were not relatively old, together with the fact that the age range of the controls was similar to that of the patients, it is unlikely that age had an effect on our observations. In control subjects, vitamin K₁ concentrations in serum can be calculated from the concentrations of apolipoproteins A₁ and B (3). However, in patients after bone fracture, the changes in serum vitamin K₁ concentrations are not paralleled with changes in their carrier systems, the lipoproteins. There is no evidence that after bone fracture apolipoprotein A₁ and B concentrations in serum are altered. It appears that serum vitamin K₁ is utilized independent of its lipoprotein carriers in serum. These observations support the concept that this vitamin is sequestered from lipoproteins in the circulation for use, perhaps at the fracture site (4). The current results indicate that the mode of sequestration of vitamin K₁ is independent of the metabolism of lipoproteins, an observation that has not previously been reported. In patients without bone fractures, the vitamin K₁ concentration can be predicted from the concentrations of apolipoproteins A₁ and B, whereas it cannot be predicted by the same equation in patients with bone fractures. The mechanism of vitamin K₁ delivery to the fracture site remains to be elucidated, but it could conceivably operate via a putative receptor in which the vitamin K₁ is selectively taken up, analogous to the interaction of HDL with cells without the loss of its apolipoprotein components. This study further indicates a need to validate the use of equations containing the serum concentrations of apolipoproteins A₁ and B to calculate vitamin K₁ concentrations in serum (3) in patient groups where changes in vitamin K₁ metabolism are in question.

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References
3. Cham BE, Smith JL, Colquhoun DM. Interdependence of serum concentra-

Molecular Forms and Ultrastuctural Localization of Prostate-specific Antigen in Nipple Aspirate Fluids, Ferdinando Mannello,1* Manuela Malatesta,2 Maurizio Sebas-
tiani,2 Serafina Battistelli,1 and Giancarlo Gazzanelli2 (1 Istituto di Istotography & Analisi di Laboratorio, Facoltà di Scienze Matematiche, Fisiche e Naturali, Università Studi, Via E. Zeppi, 61029 Urbino-PS, Italy; 2 Centro di Menologia, AUSL 1, Pesaro, Italy; * author for correspondence: fax 39-0722-322370, e-mail mannello@bio.uniurb.it)

Prostate-specific antigen (PSA) is a member of the human kallikrein family of serine proteases that for a long time was thought to be produced exclusively by the epithelial cells of the prostate gland (1). Because of its tissue specificity, PSA has been widely used as a marker for diagnosing and monitoring prostate cancer (2). However, recent studies have demonstrated the widespread distribution of PSA in a variety of tumor types, healthy tissues, and biological fluids [reviewed in Ref. (3)]. Although the physiological role and the biological significance of extraprostatic PSA currently are unknown, it has been sug-
gested that this serine protease should be regarded as a growth factor regulator produced by cells bearing steroid hormone receptors (4, 5). Several studies have biochemi-