produce changes in its concentration and in bone markers over the 3 days immediately post fracture. There is an extension to this theory that other researchers may find worthy of investigation. Vitamin B₆ is an essential cofactor for the enzyme ornithine decarboxylase, the rate-limiting enzyme in the formation of putrescine, which in turn regulates osteoblast glucose-6-phosphate dehydrogenase activity and thus osteoblast NADPH concentrations (3–6). NADPH is essential for the vitamin K cycle, in which the epoxide form of vitamin K is converted back to the naphthoquinone form, which is required for γ-carboxylation of osteocalcin (2). It is therefore possible that vitamin B₆ status could modulate the effects of vitamin K on bone metabolism.

Although the chain of events described above may seem excessively complicated, there is evidence that vitamin B₆ deficiency in rats reduces bone healing (7) and that vitamin B₆ assayed by HPLC (8) is statistically significantly lower in patients who fracture their hips in low-energy falls than in patients whose hip fractures are elective procedures (9). Further research into the interaction of these two vitamins may be indicated.

References

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Serum Cystatin C, a New Marker of Glomerular Filtration Rate, Is Increased during Malignant Progression

Cystatin C has recently been shown to be an accurate marker of glomerular filtration rate with advantages over serum creatinine (1, 2). Cystatin C, a potent inhibitor of cysteine proteases, is found mainly in extracellular fluids such as blood, cerebrospinal fluid, and seminal plasma. Its low molecular weight and stable production rate indicate that the blood concentration of cystatin C is determined mainly by glomerular filtration. The production rate of cystatin C is less altered by nonrenal factors than is the production of creatinine, and it has been reported that circulating cystatin C concentrations are not affected by inflammatory conditions or malignancy (3). Our observations, however, have revealed a significant correlation between increased serum cystatin C and malignant progression in melanoma and colorectal cancer.

In malignancy, an imbalance between cysteine proteases and their inhibitors, associated with a metastatic tumor cell phenotype, is thought to facilitate tumor cell invasion and metastasis (4). Numerous studies have provided evidence of substantial increases in mRNA, protein, and the activity of tumor cysteine proteases, accompanied by only moderately increased or unchanged concentrations of intracellular inhibitors (5). Enhanced extracellular secretion of cysteine proteases is another feature associated with tumor cell phenotype. We recently published evidence that high serum concentrations of the cysteine proteases cathepsins B and H are of prognostic importance in predicting the rate of death in colorectal (6) and melanoma cancer (7). These high concentrations
were balanced by increased serum cystatin C, which in addition to kini-
logen and α2-macroglobulin is the most important inhibitor for control-
ling the proteolytic activity of extracellular cysteine proteases. In mel-
noma we found significant increases (P = 0.02) in the cystatin C concen-
tration among patients with metastatic disease and smaller increases in
patients with primary melanoma (Fig. 1), indicating the up-regulation of
cystatin C in later events of tumor progression. In colorectal cancer, se-
rum concentrations of cystatin C were significantly increased (P <0.0001)
in patients at all Dukes stages, correlating weakly with pa-
tient age and gender (unpublished data). The correlation between cysta-
tin C and creatinine serum values (7), however, was much weaker in can-
cer patients than that reported for healthy controls (3), suggesting the
influence of nonrenal factors on the concentration of cystatin C in malign-
ant sera. The creatinine values, not significantly changed in cancer pa-
tients, suggest that patients’ renal function had not been altered at the
time of sample collection.

In our opinion the number of pa-
tients included in previous studies was too low to provide relevant in-
formation about changes in the cysta-
tin C serum concentration during malignant progression. The results
of our studies, which involved 401 patients with colorectal cancer, 97
patients with melanoma, and 124 healthy controls, strongly support
the need for further evaluation of cystatin C as a marker for glomer-
ular filtration rate determination, at least in cancer patients, to deter-
mine its potential for use in clinical practice.

References
rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. Clin
2. Finney H, Newman DJ, Gruber W, Merle P, Price CP. Initial evaluation of cystatin C mea-
surement by particle-enhanced immuno-
nephelometry on the Behring nephelometer
systems (BNA, BN II). Clin Chem 1997;43:
1016–22.
cystatin C measured by automated immuno-
4. Sloane BF. Suicidal tumor proteases. Nat Bio-
5. Lah T, Kos J. Cysteine proteinases in cancer
progression and their clinical relevance for
6. Kos J, Nielsen HJ, Krasovec M, Christensen
U, Cimerman N, Stephens RW, Brunner N.
Prognostic values of cathepsin B and carci-
oembryonic antigen in sera of patients with
colorectal cancer. Clin Cancer Res 1998;4:
1511–6.
7. Kos J, Stabuc B, Schweiger A, Krasovec M,
Cimerman N, Kopitar-Jerala N, Vrhovec I. Cath-
epsins B, H, L, their inhibitors stefin A and
cystatin C in sera of melanoma patients. Clin

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Anti-Thyrotropin Antibody Interference
in Thyrotropin Assays

To the Editor:
We read with interest the paper by
Despres and Grant (1) on antibody inter-
ference in thyroid assays. Thyroid
hormone autoantibodies, heterophile
antibodies, and rheumatoid factors are
certainly the main sources of artifacts.
As mentioned by the authors, anti-
thyrotropin (anti-TSH) antibodies are
more uncommon but may neverthe-
less deserve additional comments.

The existence of anti-TSH antibodies
in patient sera has been reported after
injections of bovine TSH (2,3). The
antibodies also appear in autoimmune
thyroid diseases such as Graves dis-
ease, Hashimoto thyroiditis, silent thy-
roiditis, and subacute thyroiditis (4–7),
and nonthyroid autoimmune disease
(6). In sera from patients with Graves
disease, the possibility that thyro-
tropin receptor antibodies (TRAbs) may
be anti-idiotype antibodies against anti-TSH antibodies or that an-
ti-TSH antibodies may be anti-idiotype antibodies against TRAbs is con-
troversial (8–12). Most of the reported anti-
TSH antibodies reacted against bovine
TSH; however, some also reacted against human TSH (4, 12–14).

The results of published studies on
anti-TSH antibody interference in TSH
assays concerned mainly RIAs. In
those cases, depending on the assay
design and the antibody specificity,
interference may yield lower or in-
creased values. Increased results were
found with the double antibody tech-
niques (4, 5, 13, 15–17). Single anti-
body techniques with polyethylene
glycol (PEG) precipitation yielded
low values (14, 15). Fewer results
have been reported with the widely
used, “sandwich” immunometric
assays (IMAs). IMA results have been
found to be lower (5) or similar to
double antibody results (6). More-
ever, different IMA kits may yield
discrepant values (14).

We previously reported (18) TSH
cancentrations that we measure (19)
with eight different third-generation
IMAs in four serum samples that con-
tained anti-TSH antibodies as
determined by increased precipitation
of protein-bound 125I bovine or human
TSH. Two samples from patients with
autoimmune thyroid disorders
(Graves disease and postpartum thy-
roiditis) contained only anti-bovine
TSH antibodies. The results of the dif-
terent TSH tests were not grossly dis-
crepant, ranging from 0.36 to 0.60
mIU/L and from 2.9 to 4.7 mIU/L for
the two samples, respectively. The
other two sera contained both anti-
bovine and anti-human TSH antibo-
dies. In the first case, our suspicion
was aroused because the high serum TSH
contrasted with an apparently healthy
clinical picture. The second case was
from a euthyroid woman who had

given birth to two children with tran-