Vitamin D and cardiovascular risk in postmenopausal women: how to translate preclinical evidence into benefit for patients

Berthold Hocher1 and Christoph Reichetzeder1

Preclinical work indicates that calcitriol restores vascular function by normalizing the endothelial expression of cyclooxygenase-2 and thromboxane-prostanoid receptors in conditions of estrogen deficiency and thus prevents the thromboxane-prostanoid receptor activation-induced inhibition of nitric oxide synthase. Since endothelial dysfunction is a key factor in the pathogenesis of cardiovascular diseases, this finding may have an important translational impact. It provides a clear rationale to use endothelial function in clinical trials aiming to find the optimal dose of vitamin D for the prevention of cardiovascular events in postmenopausal women.


A recent, large epidemiological study with a median follow-up of 8.7 years showed that the lowest quartile of 25-hydroxyvitamin D (<17.8 ng/ml) was independently associated with all-cause mortality in the general population. A similar trend for increased cardiovascular mortality was observed in the lowest quartile, but the difference was not statistically significant. However, in preselected sub-populations with an increased risk for cardiovascular diseases, including elderly people, patients with preexisting systemic inflammatory diseases such as lupus nephritis, and patients with suspected coronary heart disease, vitamin D deficiency was clearly found to be associated with cardiovascular mortality. It is of note that the effects of vitamin D or calcitriol deficiency on cardiovascular morbidity and mortality seem to be long-lasting, but they also require a long time to become obvious. We need to keep in mind that calcitriol deficiency might be dissociated from vitamin D deficiency, as vitamin D levels might be in the normal range but the conversion to calcitriol be impaired because of chronic kidney failure. Moreover, observations based on association studies such as the ones mentioned here do not prove that correction of vitamin D deficiency or insufficiency will improve clinical outcomes. Definitive proof can be obtained only by double-blind, placebo-controlled, adequately powered outcome trials.

Preclinical work in cell-culture systems and animal models helped in understanding the link between low vitamin D levels and cardiovascular disease. Vitamin D deficiency has been shown to be associated with enhanced synthesis and/or deleterious effects of many substances, including parathyroid hormone, various matrix metalloproteinases (MMPs), tumor necrosis factor-α, endothelium-derived contracting factors, and advanced glycation end products. Moreover, vitamin D deficiency also causes reduced synthesis of matrix Gla protein, osteopontin, and interleukin-10. The numerous alterations of the paracrine/endocrine system associated with vitamin D deficiency promote vascular calcification, which is one of the possible pathways involved in cardiovascular morbidity and mortality. Another pathway is left ventricular hypertrophy and impaired cardiomyocyte contractility via reduced synthesis of MMP inhibitors in the face of enhanced MMP generation and decreased synthesis of cardiac muscle proteins.

Dong et al. (this issue) report a novel molecular mechanism. In a very elegant study, they provide convincing evidence that renal artery cyclooxygenase-2 (COX-2) expression is elevated in rats with estrogen deficiency induced by ovariectomy, which in turn results in increased expression of thromboxane-prostanoid receptors. COX-2 products and thromboxane-prostanoid receptor activation reduce nitric oxide bioavailability, leading to impaired endothelium-dependent revascular relaxation. Chronic calcitriol administration is shown to restore vascular function by normalizing the endothelial expression of COX-2 and thromboxane-prostanoid receptors, thereby preventing the reduction of nitric oxide generation that is induced by thromboxane-prostanoid receptor activation. Similar effects are obtained by acute exposure to three different COX-2 inhibitors and a thromboxane-prostanoid receptor antagonist, respectively. Moreover, exposure of renal arteries from ovariectomized rats to calcitriol also improves vascular relaxation and downregulates thromboxane-prostanoid receptor expression in short-term ex vivo experiments. Finally, the attenuated nitric oxide production in aortic endothelial cells from ovariectomized rats can be restored following short-term treatment with calcitriol, a COX-2 inhibitor or a thromboxane-prostanoid receptor antagonist. The study used renal arteries as a model system for arterial}

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function. This most likely is representa-
tive of arterial function in general, but this
needs to be proven in the future.

Three questions have not been addressed
in this otherwise elegant
study with potential clinical relevance.

First, what is the optimal calcitriol dose to achieve this effect? A classical
dose-response curve is missing.

Second, does native vitamin D have the same effect as calcitriol? Keeping in
mind the well-known U-shaped vi-
tamin D concentration–mortality curve
seen in meta-analyses of association
studies,1 this question needs to be
evaluated in the future.

Third, the long-term consequences
of interfering with this newly described
pathway for the structure and function
of the kidneys and the cardiovascular
system, including mortality, need to be
carefully addressed in future animal
experiments. It would also be good to
replicate the above findings in a second,
independent animal model. These
would appear to be the first necessary
steps in translating the findings descri-
based in the landmark study by Dong
et al.7 into clinical science.

Postmenopausal women repres-
ent a clinical subgroup with high
cardiovascular risk. Hormone replace-
ment therapy with estrogen, aimed to
reduce the incidence of major cardio-
vascular adverse events, failed in this
population. It actually was found to
increase the risk, at least in women with
preexisting cardiovascular disease.

Pending additional work as suggested
above (a proper dose–response curve,
study with native vitamin D, and
replication in an independent animal
model), the pathway discovered by
Dong et al.7 could be explored in post-
menopausal women, in whom one could
examine the effect of optimal vitamin D
supplementation in a phase 2 clinical
trial before going to a large, costly phase
3 trial. Given the data provided by Dong
and co-workers,7 dose finding should be
possible by the choice of endothelial
function as a primary readout.

Hence, phase 2 trials with varying
doses considering this readout should be
used to find the optimal vitamin D
dose for reducing cardiovascular events
in postmenopausal women.

Without such an intermediary end
point in phase 2 trials, moving directly
to dose selection in a phase 3 trial
resembles navigating in fog without
radar. This happened in the past in
interventional trials aiming to demon-
strate beneficial cardiovascular effects.
They were inconclusive most likely
because of difficulties in finding the
right dose and treatment duration for
vitamin D. Figure 1 shows examples of
past experience with controlled
clinical trials in women and men aimed
to reduce cardiovascular events with
vitamin D.

In the context of the study by Dong
et al.7 it is important to note that in the
large Women’s Health Initiative study, a
randomized controlled trial in 36,282
postmenopausal women aged 50–79
years at 40 clinical sites, the combined
daily supplementation of calcium
(1000 mg) and vitamin D (10 μg) did
not alter the risk for coronary events or
stroke.8 Most likely, the dose of vitamin
D was simply too low in this study.
Moreover, the study tested two inter-
ventions at the same time without
proper understanding of the clinical
effects of calcium supplementation
alone versus vitamin D supplemen-
tation alone. Most importantly, the
authors did not use a reliable vitamin
D dose-finding tool to explore the
vitamin’s effects on cardiovascular
events in a phase 2 trial setting. They
thus took the risk of navigating through
fog without radar.

The beauty of the study by Dong
et al.7—besides the discovery of a new
pathway of vitamin D-related impair-
ment of endothelial function under
conditions of estrogen deficiency in
female rats—is that it theoretically
provides strong evidence for an end
point for dose finding in clinical phase
2 vitamin D trials. Hopefully, this study
will stimulate others to do more clinical
work in an area of unmet medical need.
Measurement of ultrasound-based
flow-mediated dilatation in the brachial

### Table 1

<table>
<thead>
<tr>
<th>Author, year, reference</th>
<th>Vitamin D group total/event n</th>
<th>Placebo group total/event n</th>
<th>Dose</th>
<th>Duration of study</th>
<th>Initial 25(OH)D levels (nmol/l)</th>
<th>Vitamin D/placebo</th>
<th>Hazard ratio for cardiovascular events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trivedi 200310</td>
<td>0.77 0.9 1.06</td>
<td>1345/477</td>
<td>2.5 mg</td>
<td>4 months</td>
<td>1341/503</td>
<td>ND/ND</td>
<td>ND</td>
</tr>
<tr>
<td>Brazier 200511</td>
<td>0.37</td>
<td>95/6</td>
<td>20 μg/d</td>
<td>1 year</td>
<td>95/5</td>
<td>18.3/17.5</td>
<td>+240</td>
</tr>
<tr>
<td>Hsia 20077</td>
<td>0.92 0.04 1.18</td>
<td>18,176/499</td>
<td>10 μg/d</td>
<td>7 years</td>
<td>18,106/475</td>
<td>ND/ND</td>
<td>n.d.</td>
</tr>
<tr>
<td>Prince 200812</td>
<td>0.25 0.83 2.73</td>
<td>151/5</td>
<td>25 μg/d</td>
<td>1 year</td>
<td>151/6</td>
<td>45.2/44.2</td>
<td>+20.3</td>
</tr>
</tbody>
</table>

**Figure 1 | Randomized, placebo-controlled intervention studies on vitamin D assessing cardiovascular risk.** Event characteristics of the analyzed studies, described by Pittas et al.7 are as follows: Trivedi et al., International Classification of Diseases, 9th Revision (ICD-9) codes for cardiovascular diseases 390.0–459.9, self-reported incidence or as cause of death from death certificate;10 Brazier et al., myocardial infarction, pulmonary edema, and atrial fibrillation (including death from cardiovascular disease), estimated from reported data;11 Hsia et al., nonfatal myocardial infarction or coronary heart disease death, from medical record review (events centrally adjudicated);8 and Prince et al., ischemic heart disease, estimated from reported data.12 ND, not done; 25(OH)D, 25-hydroxyvitamin D.
artery in clinical studies is the current gold standard for analysis of endothelial dysfunction, as it is noninvasive, is relatively repeatable and reproducible, and reflects pathophysiology. Flow-mediated dilatation seems to have cardiovascular-event predictability. Brachial artery endothelium-dependent dilatation is also significantly correlated with findings in the coronary circulation in the same patients. However, a number of new techniques have recently been proposed as potentially applicable screening tools for endothelial testing in humans, including pulse wave analysis, pulse wave velocity measurement, and pulse amplitude tonometry. Endothelial vasomotor function after reactive hyperemia by pulse amplitude tonometry, as measured in the fingertips, seems a very promising means of endothelial dysfunction analysis in humans.

In conclusion, the study by Dong et al. describes a novel pathway demonstrating that calcitriol may correct endothelial dysfunction induced by estrogen deficiency. As endothelial dysfunction is a key factor in the pathogenesis of cardiovascular diseases, the study also has a translational impact. It provides a clear rationale to use endothelial function in clinical trials aiming to find the optimal dose of vitamin D for the prevention of cardiovascular events in postmenopausal women.

**DISCLOSURE**
The authors declared no competing interests.

**REFERENCES**

**New wrinkles in old receptors: core fucosylation is yet another target to inhibit TGF-β signaling**

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Shen et al. exploit glycobiology to dampen transforming growth factor-β (TGF-β) signaling and ameliorate renal fibrosis after ureteral obstruction. Core fucosylation of N-linked oligosaccharides in TGF-β receptors is required for receptor function. Adenoviruses expressing Fut8-fucosyl transferase-shRNA inhibited receptor fucosylation, decreased tubule TGF-β signaling, and reduced fibrosis. Fut8-shRNA interferes with core fucosylation of other receptors also. Regardless, this first attempt to capitalize on a new aspect of TGF-β receptor function provides a basis for further research.


**see basic research on page 64**

Transforming growth factor-β (TGF-β) contributes to kidney disease progression by promoting fibrosis. However, there is no consensus regarding the cellular and molecular mechanisms of how renal fibrosis occurs. Possibly, the reasons include injury-related signaling by multiple ligands in several types of cells—tubules, fibroblasts, and inflammatory cells—and the complex paracrine effects of their secretory products. Several considerations suggest that TGF-β signaling in tubules is proximally situated in the chain of fibrotic events and that tubule pathology is a prime determinant of fibrosis in most renal disease. Tubules and interstitial cells situated immediately around them are intimately integrated in a process of tubulointerstitial fibrosis

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