Nitric oxide and bone

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Age-associated decrease in nitric oxide (NO) production may be related to an increase in cardiovascular events, sexual dysfunction, and osteoporosis. Relative NO deficiency is a plausible biological basis for NO replacement therapy.

Hormone replacement therapy (HRT) enhances local NO production and rectifies NO deficiency in postmenopausal women. However, excess local production of NO aggravates bone destruction in inflammatory arthropathies. In addition to its use in alleviating angina and erectile dysfunction, NO compounds could be a valuable supplemental therapy for chronic conditions including osteoporosis. Estrogen mediates its beneficial effects in bone, in part via the NO/cGMP pathway; hence NO donor therapy is an alternative to estrogen, estrogen agonists-antagonists, and androgen receptor modulator therapy in the prevention and treatment of osteoporosis. Large numbers of animal studies and human pilot studies support the concept of using NO donors for preventing bone loss. Administration of exogenous NO or prolonging endogenous NO activity are practical ways to supplement NO.

Key words: glyceryl trinitrate; cGMP; hormone replacement therapy; menopause; nitric oxide donors; nitric oxide synthase inhibitors; nitroglycerin; RANK; osteopenia; osteoporosis; bone mineral density; postmenopausal women

Introduction

Menopause-associated decreased estrogen levels increase osteoclast activity and bone turnover, resulting in bone loss.\(^1\),\(^2\) Nitric oxide (NO) has an estrogen-like beneficial effect in bone, but without estrogenic adverse effects. Therefore, NO donors could be an attractive alternative to estrogen therapy for osteoporosis.\(^3\)–\(^7\) Beneficial therapeutic effects of estrogen on bone mineral density (BMD) are in part mediated through the NO/cyclic guanosine monophosphate pathway (cGMP)\(^8\) and insulin-like growth factor (IGF-1).\(^9\)–\(^11\)

Nitric oxide has been shown to have a variety of effects on bone;\(^8\),\(^12\)–\(^23\) at medium doses, it suppressed osteoclastic bone resorption and promoted growth of osteoblasts.\(^24\),\(^25\) In aqueous buffers and culture conditions, nitrite is the principal oxidation product of NO,\(^26\) whereas in vivo NO is almost completely oxidized to nitrate. These end products have proven to be useful markers for NO biosynthesis in biological samples.\(^25\)

Nitric oxide is enzymatically produced by oxidation and cleavage of the amino-terminal nitrogen atom of amino acid L-arginine. The reaction is dependent on electrons donated by the cofactor NADPH, which requires oxygen, and yields L-citrulline as a co-product.\(^27\)

A family of three related enzymes, the NO synthases (NOS), regulates the synthesis of NO. These are characterized as a neuronal form (type 1; nNOS) originally isolated from brain; an endothelial form (type 3; eNOS) originally isolated from bovine aortic endothelial cells;\(^28\) and an inducible form (type 2; iNOS) originally isolated from murine macrophages.\(^29\) eNOS and nNOS are expressed constitutively and are characterized by highly regulated, rapid and controlled manner, but low-output NO production that imposes a tonic physiological function.\(^30\),\(^31\) Table 1 illustrates the differences and commonalities of three NOS isoenzymes.

Role of nitric oxide in bone metabolism

During the past two decades, significant advances have been made in understanding cellular mechanisms involved in bone metabolism leading to identification of novel therapeutic agents. However,
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Table 1. Chromosomal and cellular localization of NOS isoenzymes and their activities

<table>
<thead>
<tr>
<th>NOS type</th>
<th>Name</th>
<th>Chromosomal location</th>
<th>Predominant location</th>
<th>NO output</th>
<th>Calcium-dependent activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOS-1</td>
<td>nNOS (bNOS) (constitute)</td>
<td>12</td>
<td>Brain NANC neurons</td>
<td>Low</td>
<td>Dependent – Cytosolic/membrane</td>
</tr>
<tr>
<td>NOS-2</td>
<td>iNOS</td>
<td>17</td>
<td>Macrophages neutrophils</td>
<td>High</td>
<td>Independent cytosolic</td>
</tr>
<tr>
<td>NOS-3</td>
<td>eNOS (constitute)</td>
<td>7</td>
<td>Endothelium</td>
<td>Low</td>
<td>Dependent – Membrane-bound</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Smooth muscles</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aAdapted from Ref. 24.*

for proper understanding, it is important to consider multiple interactions of these chemical entities in vivo as well as during the development of new therapeutic agents. Basic interactions between cytokines, hormones, and NO in bone metabolism are illustrated in Figure 1.

Nitric oxide has been shown to regulate osteoclasts, which are responsible for bone resorption. Bone formation and resorption are key processes that are necessary for the constant remodeling that occurs in bone tissue to keep it healthy, and to repair bone micro-damage. The NO donors, have beneficial effects on controlling bone resorption (decreased osteoclastic activity), and have a milder anabolic action on bone formation (i.e., enhanced osteoblastic activity). Hence, for postmenopausal women who cannot tolerate or afford hormone replacement therapy (HRT) treatment, nitroglycerin therapy may become a cost-effective option in the future.

Furthermore, studies have demonstrated that supplementary nitroglycerin has beneficial additive effects on BMD when co-administered with vitamin D, calcitonin, and bisphosphonates. Taken together, these data suggest that long-term therapy with NO will not only increase BMD, albeit less efficiently than bisphosphonates, but may also decrease fracture rates.

Nitric oxide donor compounds, such as nitroglycerin, glyceryl trinitrate, and nitrates, are safe and cost-effective medications used in clinical practice for several decades. These compounds are well tolerated; headaches are the main adverse effect. These compounds are effective in the prevention or reversal of estrogen-depleted osteoporosis in rodent models and in human subjects. In the past, many postmenopausal women relied on HRT after menopause to reduce the risk of heart disease and osteoporosis. However, the Women’s Health Initiative (WHI) study demonstrated that, while HRT is effective in preventing fractures, it could slightly increase the risk of stroke, heart disease, and breast cancer. Thus, finding a cost-effective alternative therapy for osteoporosis with little or no adverse effects would be very useful. One such group of agents is NO donors.

In vitro cellular biological studies

Nitric oxide appears to play an autocrine and paracrine regulatory role in bone metabolism. In addition, NO modulates the activity of both osteoblasts and osteoclasts in vitro. A number of studies suggest that, NO donors increase osteoblast cell proliferation, osteocalcin synthesis, and in vitro formation of a mineralized matrix.

On the other hand, NOS inhibitors have an antiproliferative effect on osteoblastic cells in vitro and lead to enhanced bone resorption in vivo. The NO-cGMP pathway also seems to be involved in the mechanism of bone resorption by cyclosporin. Moreover, release of large amounts of NO from iNOS in cytokine-stimulated cells also has an antiproliferative effect on osteoblasts, increases osteoblast apoptosis, and enhances osteoclast-mediated bone resorption. Therefore, NO appears to have a biphasic effect on bone formations: in low concentrations, it promotes bone formation, whereas it inhibits effect at higher concentrations. Figure 2 indicates the dose-dependant effect of three NO donor compounds on osteoblast-like cell proliferation.

In primary human and rat osteoblast cultures, 17β-estradiol dose-dependently stimulates osteoblast cell proliferation and differentiation as assessed by alkaline phosphatase activity and bone nodule formation. This effect is abolished after
inhibition of NOS activity. Moreover, osteoblasts cultured from eNOS gene knockout mice do not respond to 17β-estradiol. Additionally, 17β-estradiol enhances eNOS enzyme expression and NO metabolite levels in osteoblasts. The latter are also abolished in the presence of NOS inhibitors. Collectively, these observations suggest that the stimulatory effect of estrogen on osteoblast proliferation and differentiation relies on local production of NO via stimulating the eNOS isoform.

Nitric oxide compounds have been shown to decrease bone resorption in vitro and similar effects have been demonstrated with l-arginine. Studies have demonstrated production of NO by osteoclasts in response to a rise in intracellular Ca²⁺, leading to a retraction of pseudopodia and subsequent inhibition of bone resorption. Estrogen is known to regulate eNOS in osteoblasts, and osteoblasts produce NO, whereas cytokine-induced inflammation enhances iNOS activity.

Nitric oxide is also involved in isoprenylation (geranylgeranylation) of the Rho GTPase, Rho-PK, which in turn inactivates a factor that would otherwise turn on the BMP-2/Cbfa1-Runx-2 cycle. Largely the site and rate of NO synthesis, the quantity generated, and the nature of the environment into which it is released determine these broad-ranging actions of NO and the tissue specificity. Meanwhile, the activity of NO is quenched by the presence of free hemoglobin or reactive oxygen intermediates, and the activity of antioxidant defense systems.

Nitric oxide is a mediator in mechanical stimulation

Nitric oxide is a key mediator in osteoblastic stimulation following exercise, shear-strain, and mechanical stimulation. Bone marrow cells as well as mechanical strain of bone cells produce NO. Pulsating fluid flow simulating canaliculi and shear-stress releases NO, which leads to osteoblastic stimulation. NO enhances and/or modulates the actions of the locally released cytokines.

In addition to releasing NO, fluid shear-stresses also release prostaglandins, perhaps via pre-osteoclast cells. The mechanisms involved in the transduction of the effects of mechanical forces have not been fully elucidated, but data suggest the influence of both prostaglandins and NO. Indeed, the inhibition of NO synthesis impairs the bone formation induced by mechanical loading. Furthermore, the exogenous NO donors can potentiate the osteogenic effect of loading. Upregulation of eNOS activity is critical in mechanical load-induced bone growth, as well as in fracture
healing. All data suggest that NO is involved as a second messenger in mechanical and stress-induced bone formation, and mediates the osteogenic effects of sex-steroid hormones.

NO is involved in fracture healing
There is ample evidence to show that NO is involved in fracture healing. After a fracture, a marked iNOS expression is observed within 24 hours, which is consistent with the initial inflammatory phase. iNOS was localized principally to endosteal osteoblasts, and the expression is transient. This signal seems to attract bone cells to the site of injury to activate the repair process. This is followed by increase in eNOS expression in osteocytes and in endothelial cells within the local environment. This makes sense, as the increase of blood supply is required for the repair process.

The presence of large numbers of vascular endothelial cells, and the overexpression of eNOS that generates NO locally, play a role in fracture healing. This is supported by the demonstration of time-dependent differential expression of NOS isoforms and the expression of cyclooxygenase enzymes. The above-mentioned scenarios are consistent with eNOS's being involved in the vascular response and neovascularization that are crucial to fracture repair. Moreover, the NOS inhibitors significantly impair fracture healing; this is reversed by local delivery of NO donors.

Estrogen and NO connection
The effects of estrogen in bone cells are mediated via estrogen receptor alpha, and it upregulates eNOS gene in bone. A beneficial effect of estrogen on bone is abolished in the presence of NO synthase enzyme inhibitors, such as L-NAME. This suggests that at least in part, the effects of estrogen in bone are mediated via a NO pathway. Subsequent studies demonstrated upregulation of eNOS expression and activity in human osteoblast cells after stimulation by 17β-estradiol.

A pilot human study demonstrated that nitroglycerin ointment applied once daily in oophorectomized women prevented the estrogen depletion-induced bone loss. The effect of nitroglycerin was equivalent to that of estrogen (Premarin), which prevented estrogen-deficiency-induced bone loss. This indirect evidence further supports HRT working via NO in the skeleton. These data suggest that NO therapy could serve as a safer alternative therapy in preventing postmenopausal bone loss.

Nitroglycerin has beneficial additive effects on BMD when co-administered with bisphosphonates. This is not surprising because the mechanisms of actions of these two classes of agents are different. NO donors are potential therapies to control bone loss and fragility, and long-term therapy with NO not only could increase BMD, but also may decrease fracture rates. However, the multiple interactions that affect bone cell activity and effects of various compounds in vivo, as well as the right
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Figure 3. Changes of lumbar spine BMD (predominantly tubercular bone) and femur weight (predominantly cortical bone) over the 6-week treatment period in 12-week-old female ovariectomized (OVX) Wistar rats in various treatment groups: \( E_2 = \) 17\( \beta \)-estradiol-treated; LN = \( l \) -NAME-treated, NG = nitroglycerin-treated. In vivo study demonstrated that the actions of estrogen in bone are mediated via the NO/cGMP pathway. Treatments were started immediately after ovariectomy. There was no additive effects when NO was co-administered with estrogen. Values are mean ± of SEM for five animals per group. Comparisons were made against ovariectomized rats – OVX-control. \( ^{a} P < 0.005; ^{ab} P < 0.02. \)

dosage, must be taken into account when designing clinical studies and development of new therapeutic agents.

NO and bone and joint inflammation

Enhanced expression of NO via iNOS in inflammatory conditions (e.g., lipopolysaccharide-induced bone resorption) is alleviated with NOS inhibitors. This inflammation-associated bone loss has been predominantly due to augmenting the cytokine-induced matrix metalloproteinase 1 (MMP-1) product of osteoblasts, subsequently activating osteoclasts. Nevertheless, the inhibition of osteoclast activity may be the predominant effect of NO under normal conditions. In vitro studies show that using high doses of NO also leads to rapid osteoclast cell death. NO donors also inhibit osteoclast formation in mouse bone-marrow cultures, an experimental system frequently used to study factors regulating osteoclastogenesis. The effect is likely to mediate by the NO-induced apoptosis of osteoclast progenitors that occur at physiological doses.

NO prevents ovariectomy-induced bone loss

In vivo studies suggest that the predominant effects of NOs are on osteoclast cells, but its anabolic effects on osteoblast cells are also important. Although the actions of NO on osteoblasts, osteoclasts, and osteocytes are weaker than those seen with bisphosphonates or parathyroid hormone therapy, the persistent gentle actions of NOs are likely to generate healthier and more physiological bone tissues in the longer term. The use of NOS inhibitors such as aminoguanidine or \( l \) -NAME leads to osteopenia in rats, while eNOS-deficient mice have reduced bone formation. Whereas iNOS activation enhances bone resorption, and is reported to be one of the main mechanisms of action in inflammation-induced osteoporosis. Moreover, the inhibition of NOS activity negatively affect the bone metabolism.

We have demonstrated that the NO donor nitroglycerin prevents bone loss in ovariectomy as well as corticosteroid-induced bone loss models, as assessed by BMD, bone weight, and bone histomorphometry in rats.

Figure 3 illustrates the effects of nitroglycerin on prevention of ovariectomy-induced bone loss, with NO donor therapy, in comparison to the positive control group (those receiving estrogen). Furthermore, animal models have demonstrated that almost all beneficial effects of estrogen on bone are

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block in the presence of NO synthase inhibitors such as l-NAME, 8 (Fig. 3).

Animal studies have demonstrated a similar efficacy of NO donor therapy in male rats. Among castrated rats, preservation of BMD by testosterone or estrogen is blocked by concomitant administration of the NOS blocker, l-NAME. These data suggest that NO therapy is also likely to help males, especially those with hypogonadism, as well as mitigating age-related bone loss in older males. Due to the milder nature of adverse effects, NO therapy will have a significant advantage over estrogen replacement therapy or selective estrogen receptor modulators (SERMs) in postmenopausal women, and testosterone and selective androgen receptor modulator (SARMs) in men.

Rationale for using NO donor compounds for osteoporosis

The rationale for using NO for osteoporosis has been developed after many in vitro and in vivo studies conducted over the past 20 years. 7,12,22,25,73 Postmenopausal women have low circulatory levels of NO, and this is increased following HRT administration, 74 androgen replacement (Wimalawansa, unpublished material), and calcitonin therapy. 75 Overall results from these animal and human studies indicate that nitroglycerin has wide beneficial effects on bone metabolism. A cross-sectional clinical study carried out in 1989 in 450 cardiac patients who used various doses of nitrates (arbitrarily divided into <35 mg vs. >45 mg per day) in comparison with an age- and sex-matched control group (290 persons), demonstrated a significant dose-dependent effect of NO therapy on the BMD. 25,42

Human clinical trials

Several previous clinical studies confirmed that nitrate use has favorable effects on the skeleton in postmenopausal osteoporosis. 7,76–79 For example, a recent randomized controlled clinical study reported an equivalent effect on increasing lumbar BMD after isosorbide mononitrate 20 mg daily, or with alendronate, 70 mg once a week over a 12-month period. 78 There was a 10.8% and a 12.1% increase in BMD after 12 months on isosorbide mononitrate and alendronate, respectively. The authors concluded that effects of isosorbide mononitrate are comparable to those of alendronate in improving BMD.

The first randomized human study, based on animal data, was conducted in the mid-1990s to assess the efficacy of topically administered nitroglycerin ointment in comparison with standard oral estrogen therapy (Premarin), using BMD and biochemical markers as end points. 7 Data from this pilot study established an equipotent effect of nitroglycerin to estrogen (HRT), preventing oophorectomy-induced bone loss in women (i.e., effectively preventing the early menopause-associated accelerated bone loss).

Figure 4 illustrates the efficacy as well as the equipotency of NO donor, nitroglycerin (30 mg/day) to estrogen, in maintaining the BMD in oophorectomized women. 7 While this dose is lower than that taken by cardiac patients for relief of angina, dose is almost double the one that is used in the NOVEL clinical study (taking adherence into account). Hence, the optimum dose (assuming that patients are adhering to therapy) is likely to be between 30 and 40 mg of nitroglycerin (or equivalent) per day. Figure 4B illustrates the biochemical markers urinary N-telopeptide (NTx) (bone resorption) and serum osteocalcin (bone formation) levels in this study in women treated with NO donor, nitroglycerin versus estrogen. 7

Although both drugs equally decreased the urinary NTx levels, only nitroglycerin increased serum osteocalcin and bone-specific alkaline phosphatase (BS-ALP). This supports the notion that increase in bone formation in response to nitroglycerin therapy in humans is similar to that we previously observed with animal studies. 8,12,27 Although changes in bone biomarkers were not extraordinarily high, a ∼30% decrease in urinary NTx with a 20% increase of osteocalcin and 25% increase of BS-ALP suggest a positive effect on bone balance and consequent potential to increase BMD in the longer term. 42

A cross-sectional study also supported the role of NO in enhancing BMD. 80 Adequate supplementation in animals or humans with NO precursor l-arginine could also be effective in prevention of bone losses, 81 but the amount of l-arginine necessary to inject to achieve these biological effects is high, making this approach impractical. 27 In addition, the role of combination therapies 82–84 needs to be investigated, especially with the combination of NO donors with bisphosphonates. 25
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Figure 4. Equipotency of NO donor nitroglycerin with estrogen therapy in maintaining BMD in early postmenopausal women. (A) Mean BMD (g/cm²) in lumbar spine, and (B) in the total hip in estrogen (Premarin, 0.625 mg/day) versus nitroglycerin-treated (30 mg of nitroglycerin per day) group of oophorectomized women (n = 7 per group; mean ± SEM) at baseline (open columns), at 6 months (hatched columns), and at 12 months (solid columns) of treatment. No statistical differences were observed between the two treatment groups (i.e., the responses in the two groups are comparable). (C) Changes of urinary N-telopeptide (NTx; nM BEC/mM creatinine) and (D) serum osteocalcin levels (ng/mL) in oophorectomized women treated with estrogen (Premarin®, 0.625 mg/day) versus nitroglycerin (30 mg/day) (n = 7 per group; mean ± SEM) at baseline and at 6 and 12 months.

NO dose-response, bone mineral density, and fracture reduction

A recently published case-controlled study comparing 124,655 subjects with fractures with 373,962 sex-matched controls reported that those who used (vs. not used) nitrates had a lower risk of any fracture (OR = 0.89; 95% CI = 0.86–0.92), and for hip fractures (OR = 0.85; 95% CI = 0.79–0.92); thus a 15% reduction of hip fractures was seen in those who used nitrates. This observational study further clarified that the medium doses of nitrates could be more effective than the very low or very high doses in fracture prevention.

Retrospective analysis of the Canadian MultiCenter Osteoporosis Study (CaMOS) reported that nitrate users had significantly higher BMD and lower fracture rates, but the frequency of the fractures or the doses of nitrates used were not documented. Additional, a 3-month prospective study has indicated that treatment with 20 mg isosorbide mononitrate in healthy women decreased N-telopeptide by 45.4% (95% CI = 25.8–64.9), and increased serum bone-specific alkaline phosphatase by 23.3% (95% CI = 8.9–37.8). Both actions favor bone metabolism, and suggest a degree of disassociation of bone resorption from formation. These data are similar to the previously published data.

Across many studies, intermediate doses of NO have been shown to promote skeletal health, while high doses such as those used in angina pectoris may promote bone loss. Higher doses (as with NO levels generated after iNOS activation)
lead to bone loss through osteoclast activation and osteoblast suppression. Lower-to-medium concentrations of NO (as with NO-generated via eNOS activity) stimulate osteoblast and osteocyte activity, while controlling osteoclast-mediated bone resorption.\textsuperscript{25,64}

**NOVEL clinical study**

On the basis of the pilot human study data that are described in the foregoing section, a single-center, large randomized, double-blind, placebo-controlled clinical study funded by the National Institutes of Health was conducted to assess the effectiveness of topically administered nitroglycerin in prevention of early postmenopausal bone loss. This clinical trial, known as the NOVEL [Nitroglycerin as an Option: Value in Early Bone Loss] study, was designed to answer the questions: Can nitroglycerin stop bone loss in menopausal women? If so, can this be an alternative therapy for estrogen, HRT, or SERMs? The original study was designed to compare the effects of nitroglycerin with HRT and SERM (raloxifene). Because of the WHI study data,\textsuperscript{37} the NOVEL protocol was modified to compare treatment with nitroglycerin to that with inactive ointment, calcium, and vitamin D.\textsuperscript{86,87}

This 3-year clinical study in early postmenopausal subjects evaluated the use of 22.5 mg of transdermal nitroglycerin (however, the average dose received by subjects was \(\sim 16 \text{ mg/day}\)) and calcium plus vitamin D (\(n = 88\), each group). The primary end-point, the lumbar spine BMD, was not significantly different in the patients taking nitroglycerin compared to those who received calcium plus vitamin D only. These results, together with those of the four other studies published to date, show that higher dosages should be studied.

Although the primary end point was negative, several other measurement variables demonstrated a positive trend in the group treated with nitroglycerin, in comparison to the control group. These include total body bone mineral content (DXA) \([-31.1 \text{ g} \text{ vs.} -35.3 \text{ g} (P = 0.80), \text{ percent changes} -1.3 \text{ vs.} -1.43\% (P = 0.85)]\) and the rate of decrease of BMD [the annual lumbar spine BMD decrease was 0.70\% in the active arm and 0.83\% in the placebo arm] (Fig. 5) observed over a 3-year period.

Taken together, the high adherence to calcium (630 mg) plus vitamin D (400 IU) in both groups (>85\%) and the active lifestyle of the study participants may have blunted the anticipated BMD decrease in the placebo group (expected 1.5\% vs. observed 0.8\% per year of bone loss), further decreasing the difference between the two treatment groups. Since several studies using nitrate products demonstrated positive outcomes on BMD (and/or positive biochemical markers of bone turnover), further studies are warranted using higher doses of NO donors (nitroglycerin or nitrates) in both prevention and treatment of bone loss in postmenopausal women as well as in men.

There was a somewhat lower-than-expected compliance and the dose used was considered subtherapeutic (22.5 mg of nitroglycerin a day); as a result most subjects in this clinical study applied an average of about 16 mg of nitroglycerin daily. Nevertheless, those who did adhere to the therapy demonstrated a beneficial effect that was reflected in increased BMD in relation to the biochemical marker end points.\textsuperscript{69} Nitroglycerin therapy has the potential to become a highly cost-effective treatment option for prevention and treatment of postmenopausal osteoporosis in the future.

Currently, it is not clear whether the subtherapeutic dose used, the less-than-optimal adherence to therapy, or the fact that this was conducted during the early menopausal period when a higher dose of NO is required are factors responsible for the lack of efficacy demonstrated in the NOVEL clinical study.

Nitroglycerin seems to have a relatively narrow therapeutic window for treatment of osteoporosis,\textsuperscript{3,24,25,34,64} in view of the potential lack of effects at lower dosage and a possible harm from higher dosage, perhaps the optimal dose is expected to be between 30 and 40 mg a day.\textsuperscript{24,42} The right dose is necessary to obtain positive BMD results.

**Conclusions**

We previously demonstrated that a NO donor in humans is as effective as estrogen in inhibiting oophorectomy-induced bone loss,\textsuperscript{7} suggesting an important therapeutic implication in the prevention and treatment of osteoporosis. It may be possible that additional opportunities exist for the treatment of osteoporosis, such as a NO donor in combination with an osteoclast inhibitor such as calcitonin or bisphosphonates.\textsuperscript{24,25} Since the bone turnover is relatively high during the early
postmenopausal period, it is possible that the dose of nitroglycerin required to prevent bone loss in early postmenopausal women could be higher. Moreover, different nitrate preparations have widely varying PK/PD, which must be taken into account in dose adjustments. These findings suggest that NO dose-response needs to be studied further.

The actions of NO on the skeletal system are dose-dependent and biphasic. At low concentrations (result of eNOS activity), NO stimulate osteoblast and osteocyte activity and keep the osteoclast-mediated bone resorption under control. At higher concentrations (after iNOS activation or exogenous administration of higher doses of NO donors), it may lead to bone loss. Imbalances of skeletal bioavailable NO can enhance bone turnover, bone loss, and consequent fractures. Nitric oxide can also facilitate fracture healing, is a key second messenger in mechanical stress-induced bone formation, and mediates the osteogenic-like effects of sex-steroid hormones. In view of this wide range of functions, there are multiple opportunities for therapeutic interventions using the NO-cGMP pathway for osteoporosis.

Bioavailability is likely to vary between different NO donors and nitrate preparations. The actions of NO on the skeletal system are related to dose, route, and mode and frequency of application, whereas NO insufficiency leads to bone loss. Nitric oxide has an estrogen-like effect on bone, but without estrogenic adverse effects. Therefore, NO donors could become a highly cost-effective and attractive new class of therapy for osteoporosis.

However, its therapeutic window is narrow, and hence the right dose must be given to achieve beneficial effects. Since the NOVEL clinical study data are nonconclusive, additional dose-ranging studies are necessary. Additional opportunities exist to treat osteoporosis such as administration of a NO donor in combination with an osteoclast inhibitor such as calcitonin or bisphosphonates; this may further enhance bone quality and strength in patients with osteoporosis.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Mean percent change from baseline to the given follow-up visit in BMD of the spine (A), total hips (B), femoral neck (C), and total body calcium (BMC) (D) by intention to treat analysis. Nitroglycerin-treated group; solid line, closed circles (active treatment group); calcium plus vitamin D group; dashed line, open circles (control group). There is no statistical difference between the two groups. Error bars represent standard error of the mean. The number of subjects in the active and placebo arms, respectively, are as follows: at 6 months, 92 and 90; at 12 months, 90 and 90; at 24 months, 88 and 86; at 36 months, 88 and 82.

Figure S2. On the basis of animal studies, the predicted representation of BMD responses to varying doses of nitrates and nitroglycerin in current clinical practice. This graph demonstrates the narrow therapeutic window for the use of NO donor treatments in improving human skeletal health (i.e., enhance BMD and decrease fractures).

Figure S3. Schematic global representation of the effects on the skeleton of too little and too high levels of bioavailable NO within the bone cellular environment. Either extreme can lead to increased bone turnover and decreased bone mass and bone quality, which leads to fractures.

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Conflict of interest

The author declares no conflicts of interest.

References


