Topical treatment of osteoporosis in postmenopausal women: a specific focus on nitroglycerin ointment

"There is a worldwide need for inexpensive, easily administered treatments that increase bone strength, and substantially decrease the risk of nonvertebral as well as vertebral fracture. Nitric oxide, in the form of organic nitrate may have many of these attributes."

KEYWORDS: bone geometry ■ bone remodeling ■ bone strength ■ bone turnover markers ■ cortical bone ■ nitric oxide ■ osteoporosis ■ randomized controlled trial ■ trabecular bone

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The number of osteoporotic fractures is increasing worldwide and this is accompanied by increasing disability and medical costs [1,2]. Several antiresorptive treatments, including bisphosphonates, decrease bone resorption (but also decrease bone formation), and reduce the risk of vertebral fractures, which occur in 35–70% of trabecular bone. However, nonvertebral fractures that typically occur in cortical bone, including fractures of the hip, legs, upper arms and forearms account for most of the morbidity, mortality and costs owing to fractures [3] and even the most potent antiresorptive drugs reduce the risk of nonvertebral fractures by less than a third [4–9]. Furthermore, the use of bisphosphonates has been limited by concerns about potential adverse effects of long-term use, including atypical femoral fractures and osteonecrosis of the jaw. The use of estrogen for osteoporosis has been limited by concerns regarding its adverse effects on dementia, heart disease and cancer. Teriparatide is the only therapy that increases bone formation. It reduces vertebral fracture risk by approximately 70% and may decrease the risk of a subset of very 'low trauma fractures' by 40%. However, teriparatide also increases bone resorption, is expensive, requires daily injections and its use is limited to 24 months or less [10].

There is a worldwide need for inexpensive, easily administered treatments that increase bone strength, and substantially decrease the risk of nonvertebral as well as vertebral fracture. Nitric oxide (NO), in the form of organic nitrate may have many of these attributes.

NO: effects on bone

■ Cellular & animal studies

Nitric oxide can inhibit osteoclast activity and acts as a signaling molecule in osteoblasts and osteocytes [11–19]. Furthermore, mice lacking NO synthase have defective bone formation owing to defects in osteoblast differentiation and function [20,21], indicating that NO plays an important role in regulating bone. In addition, NO donors, such as nitroglycerin (NTG) ointment, isosorbide mononitrate and isosorbide dinitrate, prevent bone loss associated with estrogen deficiency and glucocorticoid administration in rodents [13,14]. Continuous administration of nitrates induces tachyphylaxis to their effects on bone [15].

■ Human studies

An observational study conducted by our group using data from the Study of Osteoporotic Fractures indicated that older women taking nitrates intermittently for angina have higher BMD at the femoral neck compared with nonusers and women taking it continuously [16]. Another study suggested that women taking nitrates have a lower risk of all fractures including hip fractures [17]. Both of these studies included several preparations of nitrates such as oral, sublingual and topical NTG ointment suggesting that the preparation is not critical to the effects on the bone. That said, preliminary work by our group indicates that headaches, a common adverse event with nitrates, are less common with topical than oral preparations. Furthermore, a topical preparation, such as NTG ointment may be preferable in specific populations, including those who complain of gastrointestinal symptoms with oral medications or those who are averse to injections (subcutaneous or intravenous).

A total of three studies have reported on the use of topical NTG ointment in postmenopausal osteoporosis. The first was an open label trial

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that randomized 16 oophorectomized women, aged 36–45 years, to NTG ointment (15 mg) applied once daily or oral conjugated estrogen (0.625 mg/day) [14]. After 6 months, women taking NTG ointment had a 40% decrease in *N*-telopeptide and 25% increase in bone specific alkaline phosphatase compared with baseline. The second was a recently published randomized trial of once daily NTG ointment (Nitro-Bid 22.5 mg), which did not find increased BMD at the lumbar spine, femoral neck of total hip, however adherence to treatment was poor [18].

> *"Our work suggests that, in contrast to the currently available therapies, nitroglycerin uncouples turnover."*

In the third and most recent study, published by our group, 243 postmenopausal women with BMD T-scores between 0 and -2.0 at the lumbar spine were randomly assigned to receive 15 mg of NTG ointment or placebo daily at bedtime for 24 months [19]. We examined the effects of NTG on bone turnover, bone density, bone geometry and strength, 81% of women adhered to at least 95% of the doses and complete follow-up measurements were obtained for 94%. Compared with placebo, NTG increased spine BMD 6.7% (95% CI: 5.2–8.2; $p < 0.001$), femoral neck BMD 7.0% (95% CI: 5.5–8.5%; $p < 0.001$) and total hip BMD 6.2% (95% CI: 5.2–7.3; $p < 0.001$) at 2 years. Treatment with NTG also increased cortical thickness (13.9 and 24.6%), cortical area (10.6 and 10.0%) and periosteal circumference (7.4 and 2.9%) at the radius and tibia respectively. This pattern indicates that NTG increases cortical bone mass; the increase in periosteal diameter suggests that it may induce formation of new bone on the periosteal surface, a biologically unique effect not observed with antiresorptives. As would be expected from these effects on cortical bone, NTG also increased indices of bending and twisting strength: polar section modulus (10.7 and 9.8%) and polar moment of inertia (7.3 and 14.5%) at the radius and tibia, respectively. We also found that NTG ointment uncoupled bone formation from bone resorption: it increased bone specific alkaline phosphatase by 36% and decreased *N*-telopeptide levels by 51% at 2 years ($p < 0.001$). The only significant adverse event associated with topical NTG use was complaint of headaches. During a 1-week run-in phase with 15 mg of NTG ointment, 104 of the 400 women stopped treatment owing to headache. Among those who continued into the randomized trial, seven in the NTG and two in the placebo group stopped treatment during the 2 years owing to headaches. Although 36% of women receiving NTG reported a headache during the first 6 months of the trial, headache was uncommon by 2 years (1.7% in the NTG vs none in the placebo group).

> *"In addition to the unique mechanism of action, nitroglycerin has several advantages over the currently available treatments."*

Conclusion & future perspective

Our work suggests that, in contrast to the currently available therapies, NTG uncouples turnover. It increases markers of bone formation and decreases markers of bone resorption. Bisphosphonates, estrogens, raloxifene and denosumab decrease bone resorption and bone formation [5–8]. Teriparatide also retains the coupling of turnover with increases in formation accompanied by increases in resorption [10]. We also found that the differential effects of NTG on bone turnover increases with time, suggesting that its efficacy continues or even increases over 2 years of use. Teriparatide causes an initial peak in bone formation and a dip in bone resorption, however, these effects wane after 3 or 4 months suggesting that its efficacy may diminish over long-term use [10]. While there are no direct comparisons, our results suggest that NTG ointment results in greater increases in cortical thickness at the tibia a 22.5% increase from baseline over 2 years than alendronate (3% over 2 years), denosumab (5% over 1 year) and teriparatide (1.5% over 1 year) [22–24]. In addition, the increase in periosteal circumference strongly suggests that NTG stimulates periosteal apposition of bone. Increased periosteal circumference increases bone size producing greater resistance to applied forces, particularly in bending and torsion. Moreover, increased bone size at the femur or wrist protects against fractures independent of increased BMD [25,26]. The changes in cortical bone geometry caused by NTG ointment may translate into an important reduction in fractures – nonvertebral fractures, such as hip fractures, in particular – that might be greater than the risk reduction observed with the currently available osteoporosis treatments.

In addition to the unique mechanism of action, NTG has several advantages over the currently available treatments. Nitrates are inexpensive and widely available. Furthermore, the fact that this agent can be used topically eliminates the possibility of gastrointestinal side effects, it can be

administered without regard to meals and other medications, and it can be utilized in patients who cannot comply with the requirements of administration of bisphosphonates (those who have difficulty swallowing or those who cannot remain seated upright). Finally, it might be a reasonable option for patients who are averse to injections (subcutaneous or intravenous). The efficacy of nitrates for reducing risk of fracture remains to be tested in a larger randomized controlled trial.

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Bibliography

1 Cummings SR, Kelsey JL, Nevitt MC, O'Dowd KJ. Epidemiology of osteoporosis and osteoporotic fracture. *Epidemiol. Rev.* 7, 178–208 (1985).

2 Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. *Osteoporos. Int.* 17(12), 1726–1733 (2006).

3 Cummings SR. A 55-year-old woman with osteopenia. *JAMA* 6296(21), 2601–2610 (2006).

4 Black DM, Cummings SR, Karpf DB *et al.* Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Fracture intervention trial research group. *Lancet* 348, 1535–1541 (1996).

5 Cummings SR, Black DM, Thompson DE *et al.* Effect of alendronate on risk of fracture in women with low bone density but without vertebral fractures: results from the fracture intervention trial. *JAMA* 280(24), 2077–2082 (1998).

6 Harris ST, Watts NB, Genant HK *et al.* Effects of risedronate treatment on vertebral and nonvertebral fractures in women with postmenopausal osteoporosis: a randomized controlled trial. Vertebral Efficacy with Risedronate Therapy (VERT) study group. *JAMA* 282(14), 1344–1352 (1999).

7 Black DM, Delmas PD, Eastell R *et al.* Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. *N. Engl. J. Med.* 3356(18), 809–1822 (2007).

8 Cummings SR, San Martin J, McClung MR *et al.* Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N. Engl. J. Med.* 20361(8), 756–765 (2009).

9 Ettinger B, Black DM, Mitlak BH *et al.* Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. Multiple outcomes of raloxifene evaluation (MORE) investigators. *JAMA* 282(7), 637–645 (1999).

10 Neer RM, Arnaud CD, Zanchetta JR *et al.* Effect of parathyroid hormone (1–34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *N. Engl. J. Med.* 344, 1434–1441 (2001).

11 Chae HJ, Park RK, Kang JS *et al.* Effect of stem cell factor, interleukin-6, nitric oxide and transforming growth factor-beta on the osteoclast differentiation induced by 1 α,25-(OH)2D3 in primary murine bone marrow cultures. *Pharmacol. Toxicol.* 82(5), 223–229 (1998).

12 Evans DM, Ralston SH. Nitric oxide and bone. *J. Bone Miner. Res.*11(3), 300–305 (1996).

13 Wimalawansa SJ, Chapa MT, Yallampalli C, Zhang R, Simmons DJ. Prevention of corticosteroid-induced bone loss with nitric oxide donor nitroglycerin in male rats. *Bone* 21(3), 275–280 (1997).

14 Wimalawansa SJ, De Marco G, Gangula P, Yallampalli C. Nitric oxide donor alleviates ovariectomy-induced bone loss. *Bone* 18(4), 301–304 (1996).

15 Wimalawansa SJ, Chapa T, Wimalawansa S, Fang L, Yallampalli C. Dose and frequency effects of nitric oxide donor nitroglycerine on bone. Presented at: *79th Annual Meeting of the Endocrine Society*. MN, USA, 11–14 June (1997) (Abstract 3–248).

16 Jamal SA, Browner WS, Bauer DC, Cummings SR. Intermittent use of nitrates increases bone mineral density: the study of osteoporotic fractures. *J. Bone Miner. Res.* 13(11), 1755–1759 (1998).

17 Rejnmark L, Vestergaard P, Mosekilde L. Decreased fracture risk in users of organic nitrates: a nationwide case–control study. *J. Bone Miner. Res.* 21(11), 1811–1817 (2006).

18 Wimalawansa SJ, Grimes JP, Wilson AC, Hoover DR. Transdermal nitroglycerin therapy may not prevent early postmenopausal bone loss. *J. Clin. Endocrinol. Metab.* 94(9), 3356–3364 (2009).

19 Jamal SA, Hamilton CJ, Eastell R, Cummings SR. Effect of nitroglycerin ointment on bone density and strength in postmenopausal women: a randomized trial. *JAMA* 305(8), 800–807 (2011).

20 Aguirre J, Buttery L, O'Shaughnessy M *et al.* Endothelial nitric oxide synthase gene-deficient mice demonstrate marked retardation in postnatal bone formation, reduced bone volume, and defects in osteoblast maturation and activity. *Am. J. Pathol.* 158(1), 247–257 (2001).

21 Armour KE, Armour KJ, Gallagher ME *et al.* Defective bone formation and anabolic response to exogenous estrogen in mice with targeted disruption of endothelial nitric oxide synthase. *Endocrinology* 142(2), 760–766 (2001).

22 Burghardt AJ, Kazakia GJ, Sode M, de Papp AE, Link TM, Majumdar S. A longitudinal HR-pQCT study of alendronate treatment in post-menopausal women with low bone density: relations between density, cortical and trabecular micro-architecture, biomechanics, and bone turnover. *J. Bone Miner. Res.* 1825(12), 2267–2528 (2010).

23 Macdonald HM, Nishiyama KK, Hanley DA, Boyd SK. Changes in trabecular and cortical bone microarchitecture at peripheral sites associated with 18 months of teriparatide therapy in postmenopausal women with osteoporosis. *Osteoporos. Int.* 22(1), 357–362 (2011).

24 Seeman E, Delmas PD, Hanley DA *et al.* Microarchitectural deterioration of cortical and trabecular bone: differing effects of denosumab and alendronate. *J. Bone Miner. Res.* 25(8), 1886–1894 (2010).

25 Ahlborg HG, Johnell O, Turner CH, Rannevik G, Karlsson MK. Bone loss and bone size after menopause. *N. Engl. J. Med.* 24349(4), 327–334 (2003).

26 Black DM, Bouxsein ML, Marshall LM *et al.* Proximal femoral structure and the prediction of hip fracture in men: a large prospective study using QCT. *J. Bone Miner. Res.* 23(8), 1326–1333 (2008).