

2011

Nitric Oxide and Osteoporosis: What a Gas!

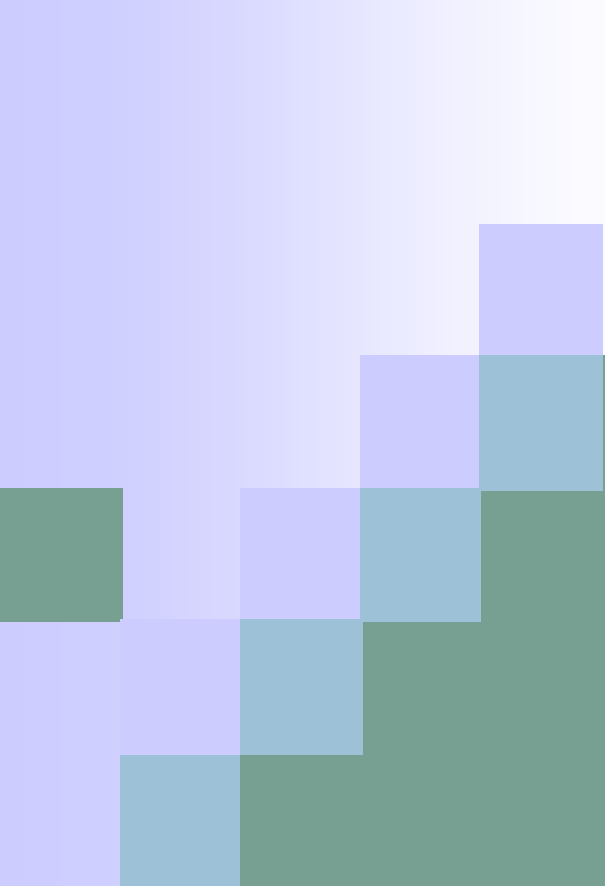
Sophie Jamal
University of Toronto

Follow this and additional works at: <http://ir.lib.uwo.ca/ccaapres>

 Part of the [Geriatrics Commons](#)

Citation of this paper:

Jamal, Sophie, "Nitric Oxide and Osteoporosis: What a Gas!" (2011). *Canadian Centre for Activity and Aging Presentations*. 2.
<http://ir.lib.uwo.ca/ccaapres/2>



Nitric Oxide and Osteoporosis: What a Gas!

Sophie Jamal, MD, PhD, FRCPC
Associate Professor of Medicine
University of Toronto



Outline

- Pathophysiology of osteoporosis
- Nitric oxide and bone turnover
- The effects of isosorbide mononitrate on markers of bone turnover
- The effects of nitroglycerin on bone turnover geometry and strength

Effect of estrogen and transdermal NTG on BMD (Wimalawansa et al Bone 1996)

Treatment group	Percent increase in BMD (L2-L4) over 6 weeks
Sham operated	25% ± 2%
Ovariectomized rats	8% ± 3%
Ovariectomized + Estrogen	27% ± 5%*
Ovariectomized + NTG	20% ± 3%**
Ovariectomized + E + NTG	22% ± 2%*

*different than ovariectomized rats at p<0.005

** different than ovariectomized rats at p<0.02



Low bone mineral density (BMD): a risk factor for osteoporotic fractures

- Relationship between hip fracture and hip BMD: 2.6 RR/SD
- BMD = bone formation - bone resorption
- Increased bone resorption at menopause
- Nitric oxide may contribute to postmenopausal bone loss

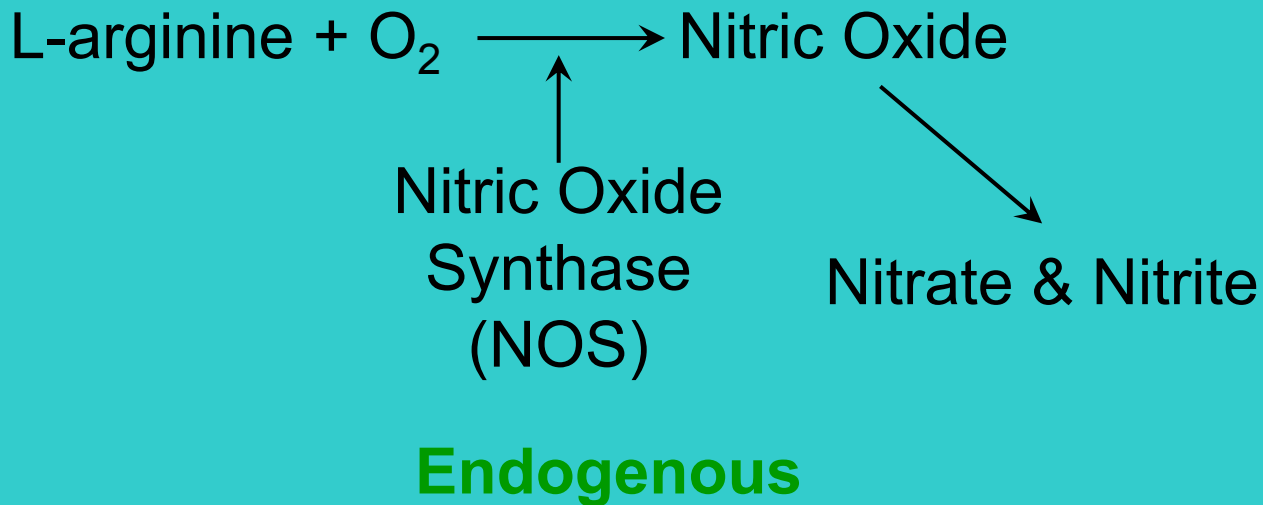
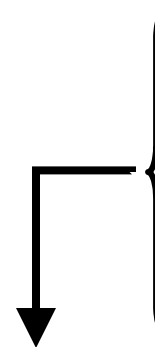
Sources of nitric oxide

Exogenous

Isosorbide Mononitrate

Isosorbide Dinitrate

Nitroglycerin





Bone cells make nitric oxide

- Two cell types involved in bone remodeling:
 - Osteoclasts → bone resorption
 - Osteoblasts → bone formation
- Osteoblasts produce nitric oxide synthase
- Estrogen and mechanical strain produce nitric oxide synthase



NO influences osteoclasts

- Nitric oxide has a biphasic effect on osteoclasts

- Low levels enhance osteoclast activity and differentiation

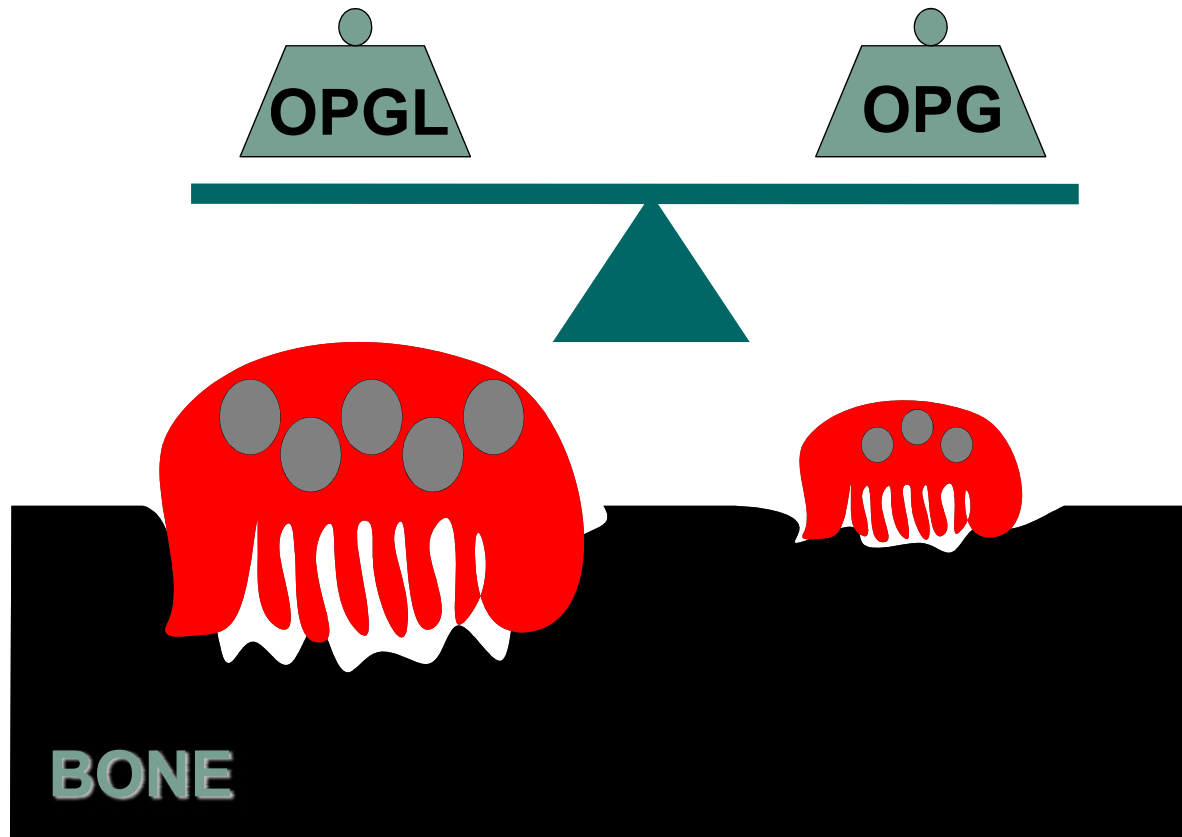
- High levels inhibit osteoclast activity and differentiation



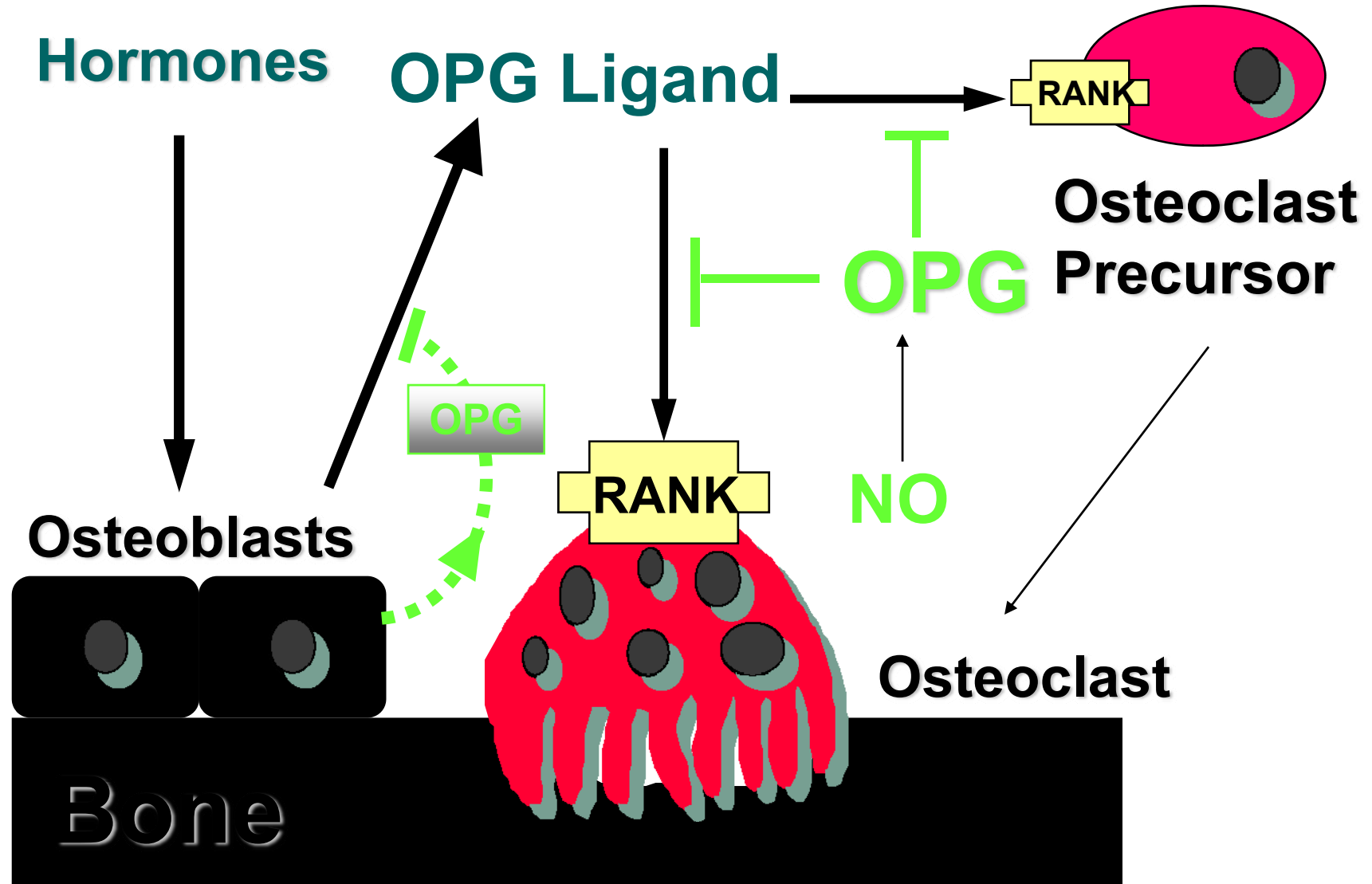
NO may mediate effects via OPG

- High levels of nitric oxide activate OPG
- Low levels of nitric oxide activate OPGL

Bone resorption depends on the balance of OPG and OPGL



The OPG/OPGL/RANK axis





What about osteoblasts?

- Limited data
- Biphasic activity:
 - High levels of NO stimulate osteoblast activity
 - Low levels of NO inhibit osteoblast activity



In vitro data: a summary

- Nitric oxide stimulates OPG
 - OPG binds to OPGL
 - Prevents binding of OPGL to RANK
 - Decreased osteoclast activity (bone resorption)
- Issues
 - Is the effect similar with nitric oxide donors?
 - Does the decrease in bone resorption lead to increased bone mineral density?

Effect of estrogen and transdermal NTG on BMD (Wimalawansa et al Bone 1996)

Treatment group	Percent increase in BMD (L2-L4) over 6 weeks
Sham operated	25% ± 2%
Ovariectomized rats	8% ± 3%
Ovariectomized + Estrogen	27% ± 5%*
Ovariectomized + NTG	20% ± 3%**
Ovariectomized + E + NTG	22% ± 2%*

*different than ovariectomized rats at p<0.005

** different than ovariectomized rats at p<0.02

Effect of frequency of administration

Treatment Group (n = 5)	Change in Spine BMD
Sham operated	6.3 % ± 5.3 *
Ovariectomy (OVX)	- 2.5 % ± 2.0
OVX + estrogen	5.9 % ± 3.4*
OVX + 0.2 mg NTG once a day	6.2 % ± 2.8*
OVX + 0.2 mg NTG twice a day	1.9 % ± 2.1
OVX + 0.2 mg NTG three times a day	- 0.2 % ± 3.3



Does NO play a role in menopause?

- Menopause is characterized by:
 - Increased bone resorption
 - Low circulating estrogen
 - Low levels of nitric oxide
- Treatment with HRT increases nitric oxide and decreases bone resorption

Nitrates and bone mineral density - 1996

- SOF study (n = 6201) (Jamal SA et al JBMR 1998)
 - BMD at heel and spine
 - Daily users = nonusers
 - Intermittent users higher
- Open label trial (n = 16) (Wimalawansa SJ, JBMR 2000).
- Conjugated estrogen or transdermal nitroglycerin
- Equal BMD at one year

Percent difference in BMD, nitrate users compared with nonusers

Daily vs. Nonusers (n = 317, 5810) (95% CI)	Intermittent vs. Nonusers (n=74,5810) (95% CI)
--	---

Hip BMD

Unadjusted	0 (-2.7 to 1.4)	0 (-4.1 to 4.1)
Adjusted	1.3 (0.14 to 4.1)	2.6 (0.4 to 6.8)

Heel BMD

Unadjusted	-2.6 (-5.3 to 0)	0 (-5.3 to 7.9)
Adjusted	0 (-2.6 to 2.6)	5.3 (2.6 to 11)

Nitrates and Fractures

- 124,655 subjects with fractures (cases)
- 373,962 controls
- Self-reported nitrate use 1995-2000
- Nitrate use associated with decreased fracture risk:
 - Any fracture: OR = 0.89 (0.86 to 0.92)
 - Hip fracture: OR = 0.85 (0.79 to 0.92)



Findings so far...

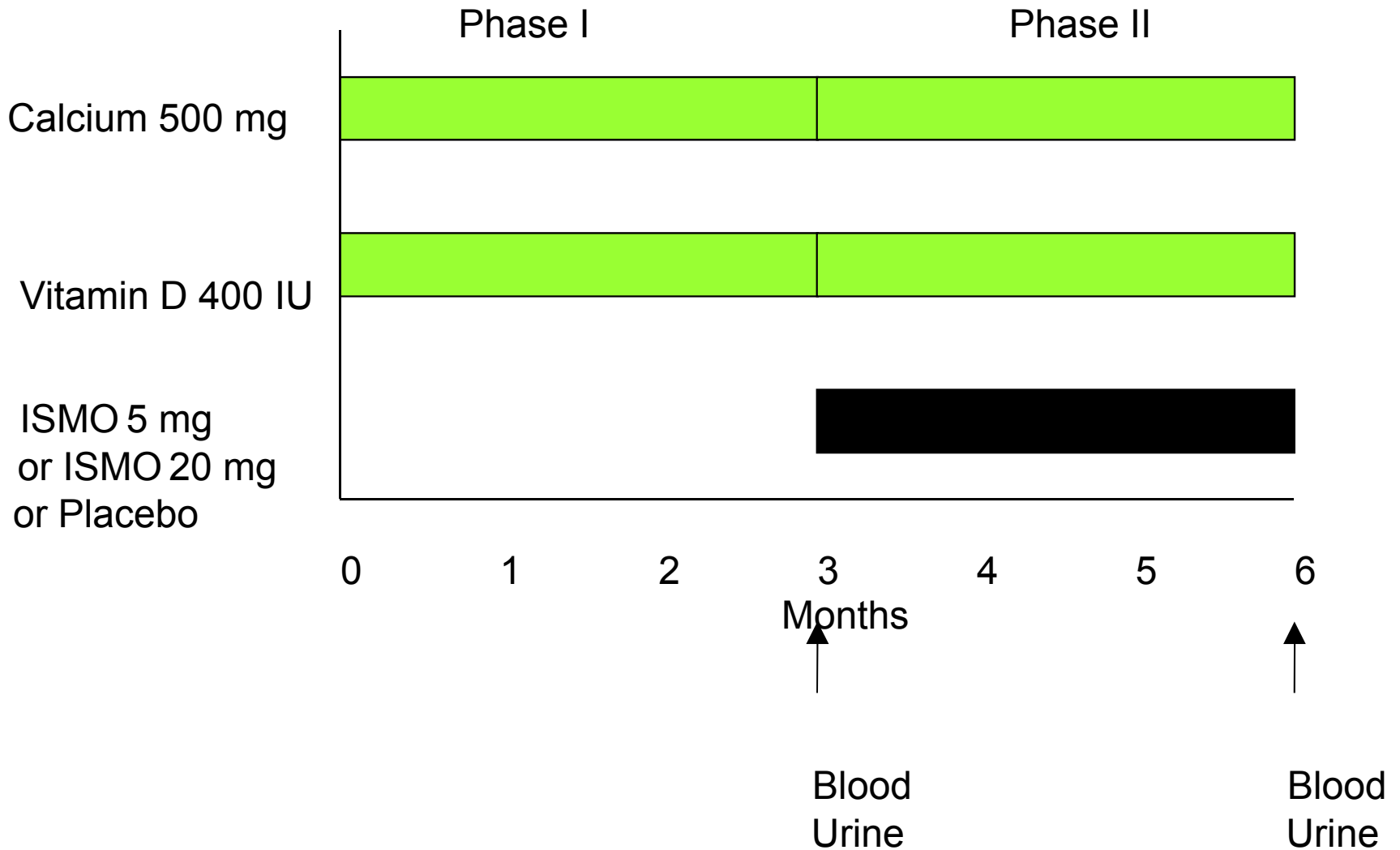
- Use of nitrates may be associated with increases in BMD
- Use of nitrates may decrease risk of fractures
- Tolerance to nitrates might exist



The second study- 1998

- To determine the effects of isosorbide on markers of bone turnover
- To assess if there is a dose response
- Applied for funding 1998, received funding 1999
- Completed study 2002
- Published 2004

Study design





Study subjects

- Inclusion Criteria:

- Postmenopausal women, 50 to 80 yrs
- Osteopenia or normal BMD at femoral neck

- Exclusion Criteria:

- Low trauma fracture (hip, wrist, spine)
- Active bone disease
- Treatment for osteoporosis
- Treatment with steroids
- Heart disease



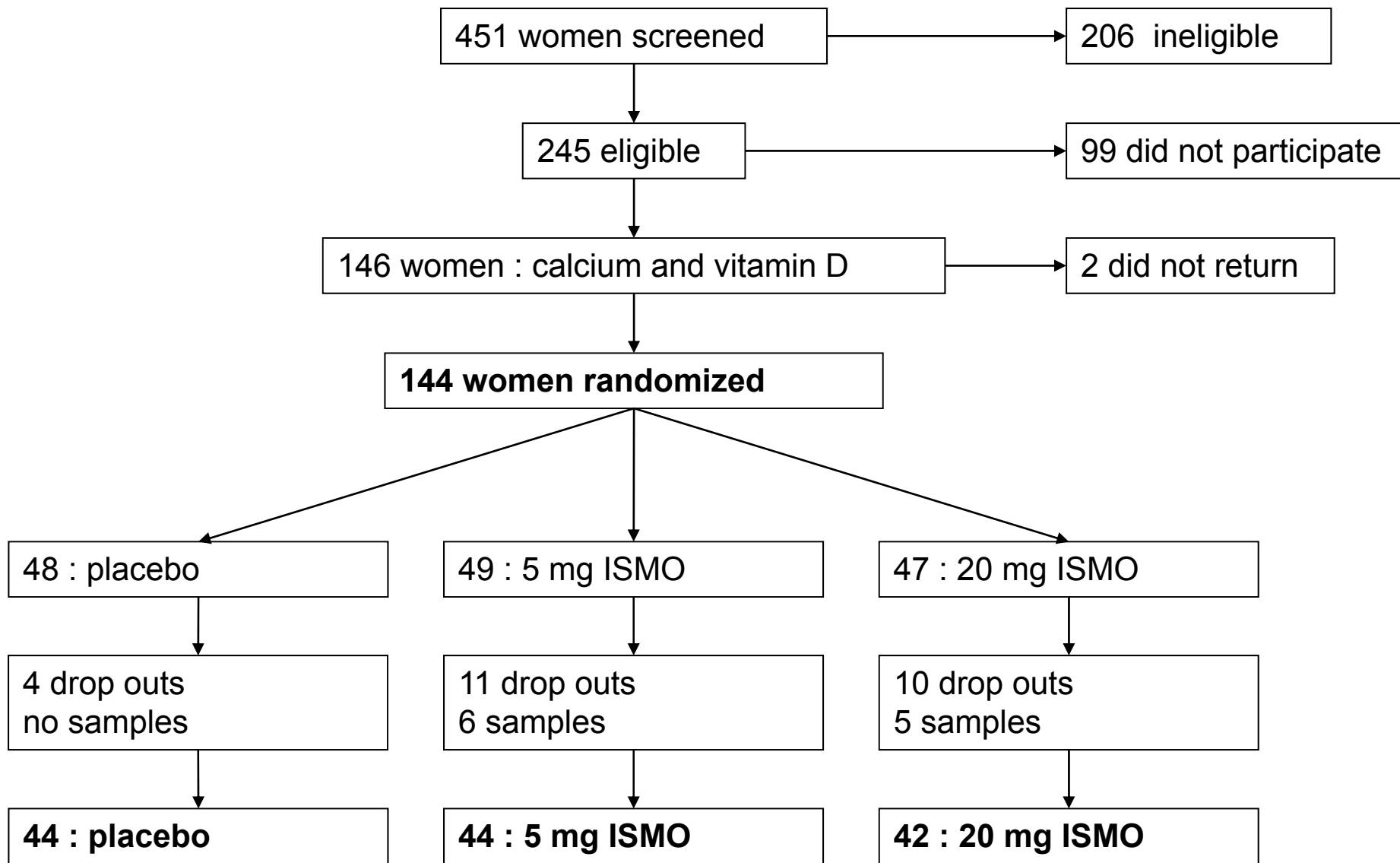
Markers of bone turnover

- 2 classes of biochemical markers:
 - Bone formation (osteoblast activity)
 - Bone resorption (osteoclast activity)
- Resorption and formation are coupled
- May be different with ISMO



Why biochemical markers?

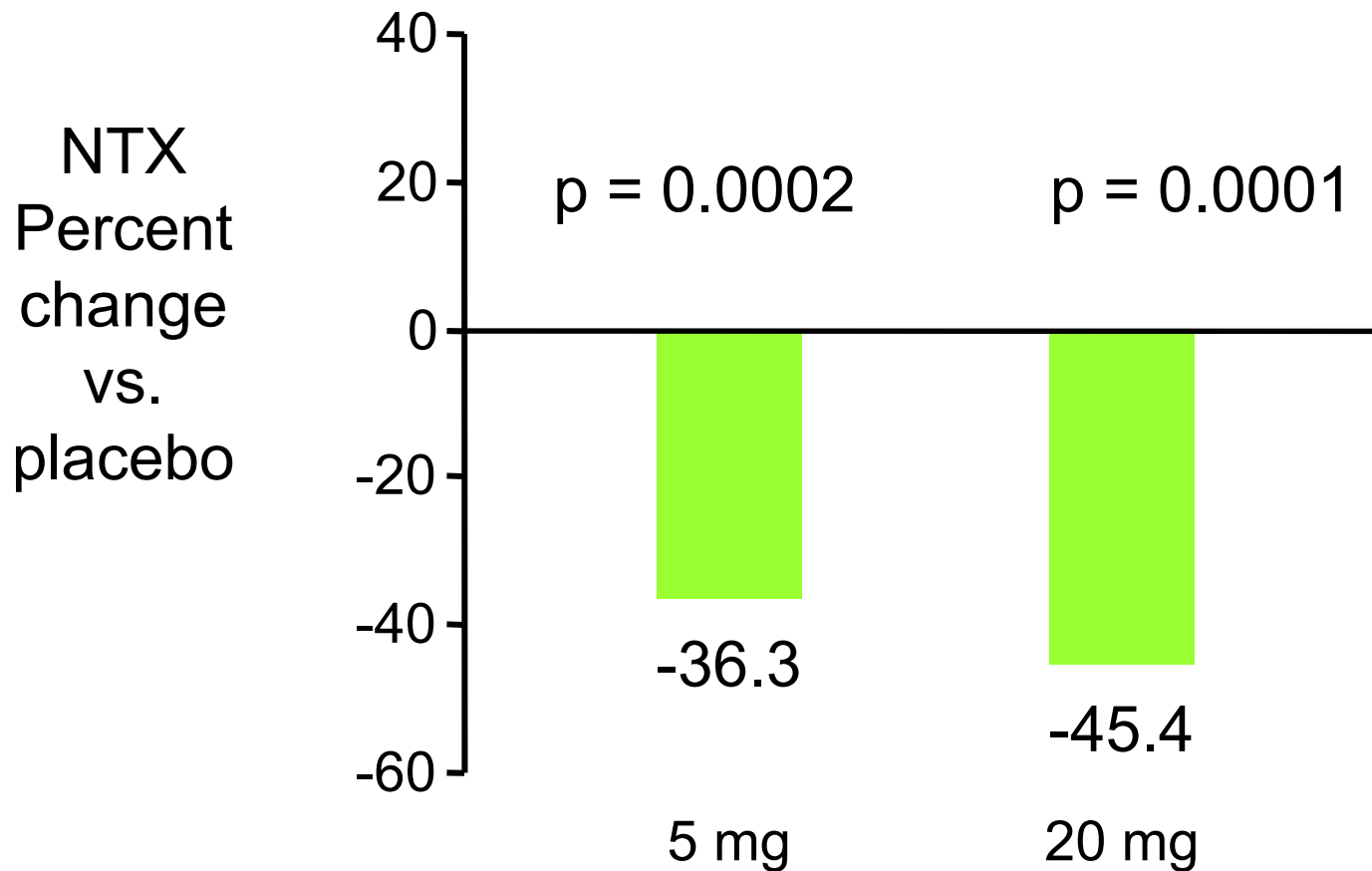
- Markers of bone turnover are correlated with bone mineral density
- Substantial changes in markers can be seen within 3 months of treatment
- Minimizes costs
- Minimizes adverse events



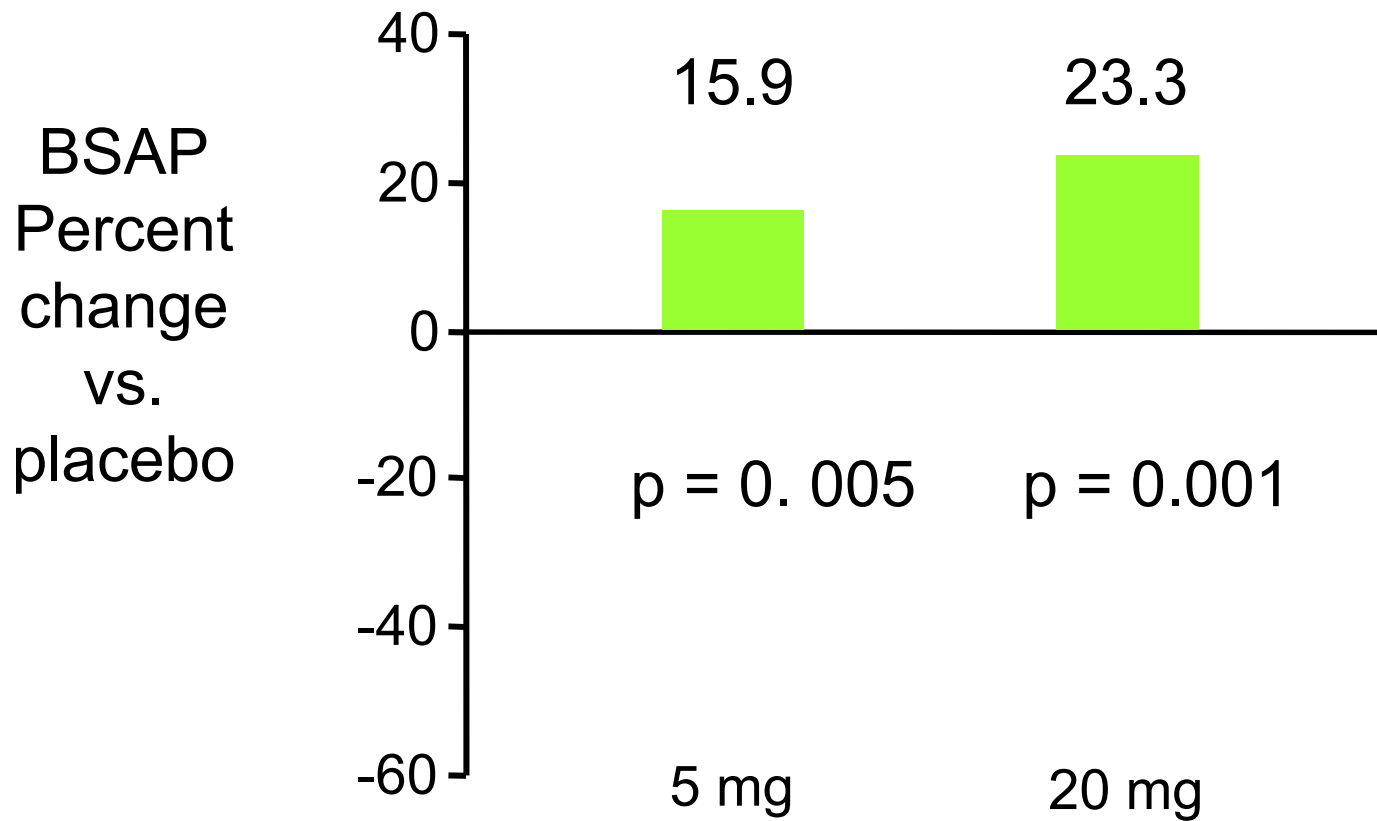
Subject characteristics

	Placebo (n = 48)	5 mg ISMO (n = 49)	20 mg ISMO (n = 47)
Age (yrs)	62 ± 7.0	59 ± 7.5	59 ± 5.5
Weight (kg)	74 ± 16	70 ± 15	74 ± 15
Caucasian	45 (94%)	44 (90%)	44 (94%)
Current smoker	5 (10%)	5 (10%)	7 (15%)
Years since menopause	13 ± 8	12 ± 10	10 ± 6
NTX (nmol BCE/mmol BSAP)	62 ± 65.9	79 ± 61.8	82 ± 71.5
BSAP (IU/L)	25 ± 6.9	23 ± 6.3	22 ± 6.6

ISMO decreased bone resorption markers



ISMO increased bone formation markers



Headaches were common

	Placebo (n = 48)	5 mg ISMO (n = 49)	20 mg ISMO (n = 47)
Discontinued study	4	11	10
D/C due to headache	2	7	9



Limitations

- Generalizability
 - Study population healthy Caucasians
- Duration of study was 12 weeks
- Studies with BMD and fracture end points are needed

Implications of our findings

- Decrease in NTx similar to bisphosphonates
- 40% decrease in NTx: 30% decrease in fracture risk
- We also found an increase in formation markers-
- ?greater fracture reduction than with antiresorptives
- Headache may limit the use of this drug:
 - 10% of women had headaches, 75% of drop outs
 - 4X more common in women randomized to ISMO
 - Higher than reported in CV literature
 - ?Less headaches with transdermal NTG

The third study

- Applied for funding March 2004- rejected
- Reapplied for funding March 2005
- Funded October 2005
- Completed Trial March 2010
- 2 part randomized controlled trial:
 - 3 week cross over study to compare tolerability of NTG and ISMO
 - 2 year study of best tolerated nitrate
- Outcomes in 2 year study:
 - Change in lumbar spine and hip BMD
 - Change in bone turnover markers
 - Changes in trabecular bone with pQCT



Study Hypothesis

15 mg of Nitroglycerin, applied once daily, will lead to uncoupling of bone turnover, increases in bone mineral density and bone geometry, compared with placebo.



Study Design

- One week run in phase
- 24 month duration
- End points:
 - Bone turnover markers
 - Bone mineral density
 - pQCT



Study Subjects

- Inclusion:

- Postmenopausal ≥ 50 years
- L spine T score between 0 and -2.0

- Exclusion:

- Migraine headaches
- Already taking nitrates
- SBP ≤ 100 mm Hg, DBP ≥ 110 mm Hg



Statistical Analyses

- Primary Endpoint:
 - Lumbar spine BMD

- Secondary Endpoints:
 - Hip BMD
 - pQCT at radius and tibia
 - B-ALP, NTX (log transformed data)

Assessed for eligibility (n=1526)

Excluded (n = 1283)
Did not meet inclusion criteria (541)
Declined to participate (585)

Enrolled in 1 week run in phase (n = 400)

Discontinued study (n= 157)
Headaches (93)
Headaches and nausea (11)

Randomized (n = 243)

Nitroglycerin (n =126)

Placebo (n = 117)

Discontinued intervention (n = 30)
Headaches (n =7)
No follow up data (n = 8)

Discontinued intervention (n =15)
Headaches (n = 2)
Lost to follow up (n = 6)

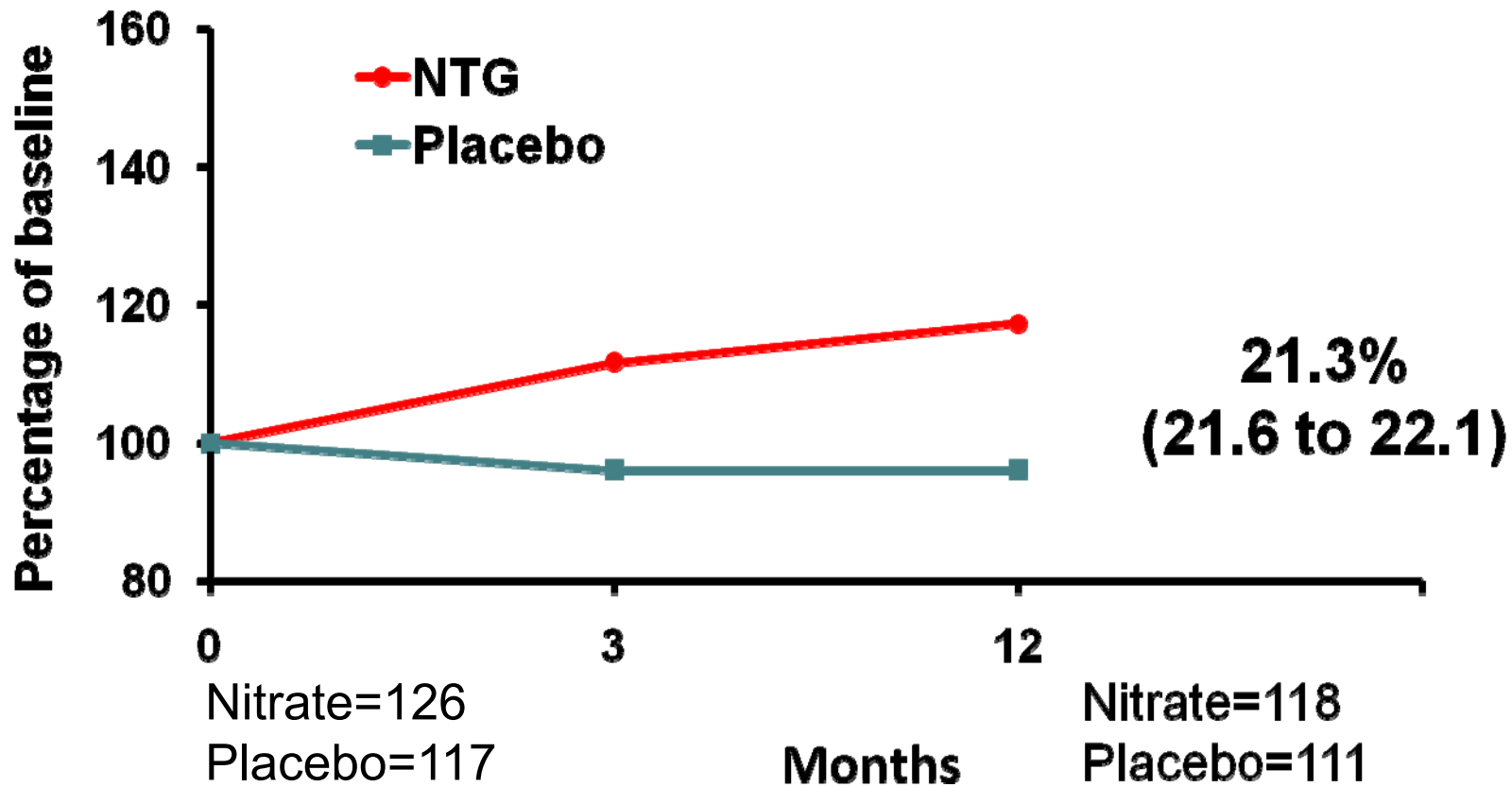
Analysed:
Data on 116 at 24 m

Analysed:
Data on 109 at 24m

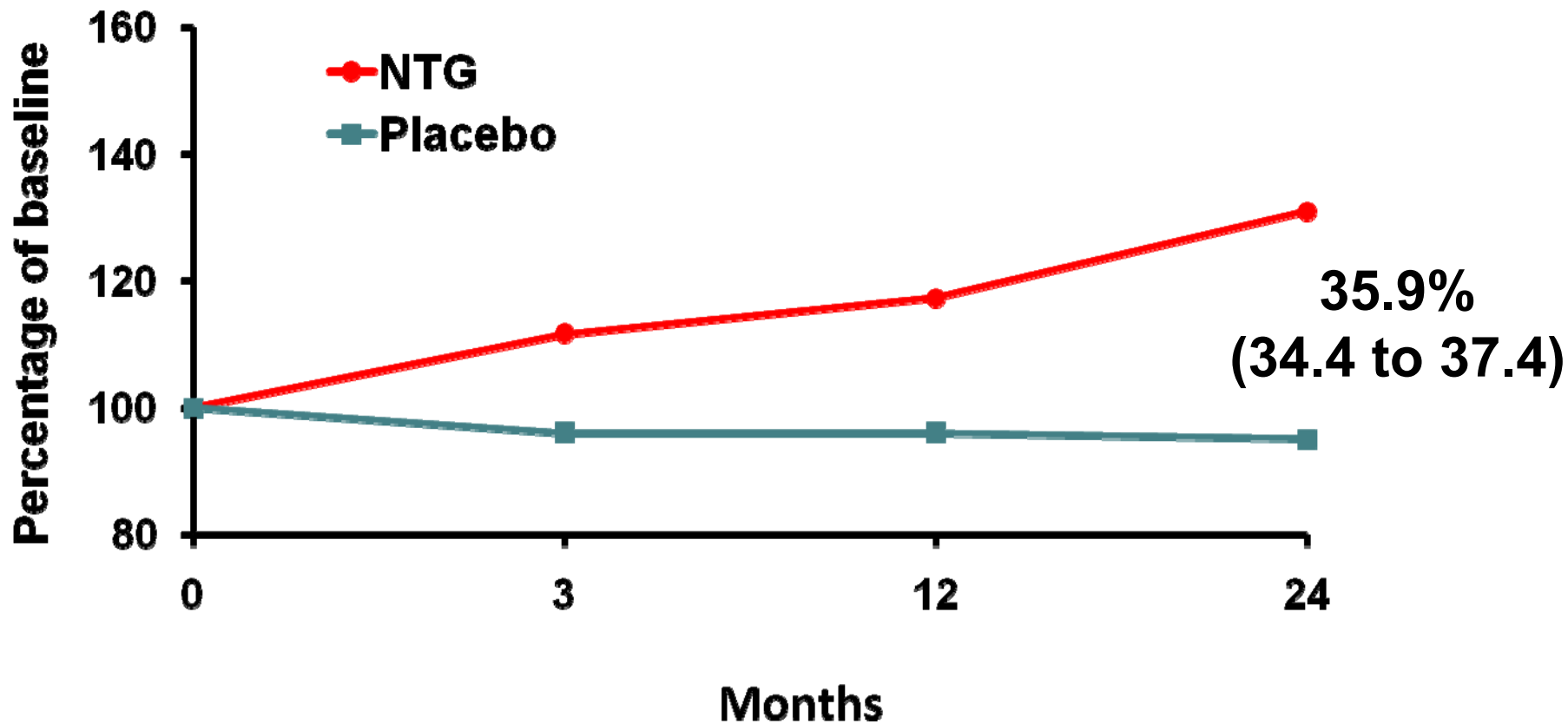


	NTG Ointment N = 126	Placebo N = 117
Age (yrs)	61.3	61.9
Weight (kg)	70.3	70.9
Vitamin D (IU/day)	783.2	753.2
Calcium (mg/day)	1548.8	1565.6
L Spine T-score	-0.9	-1.1

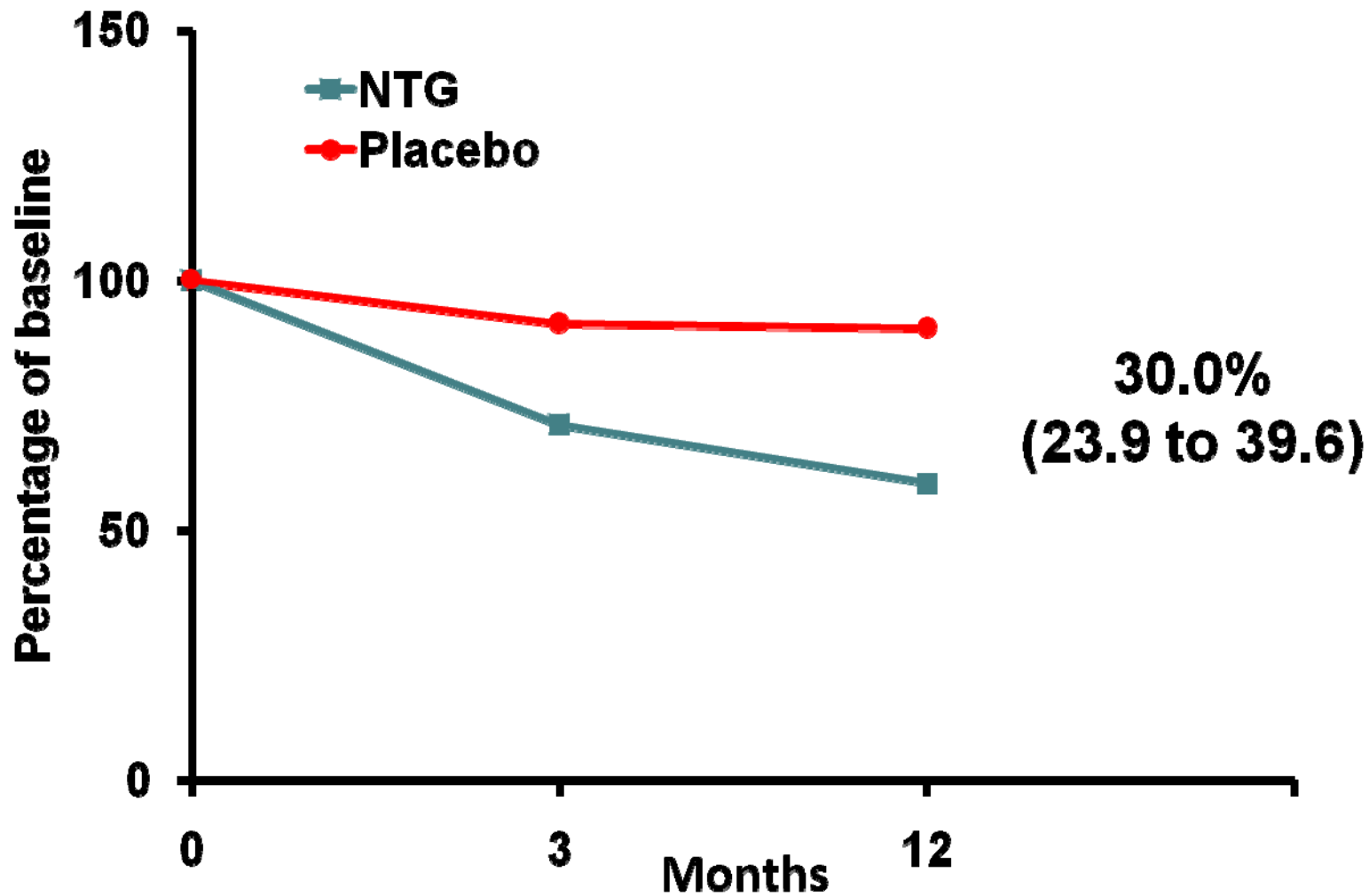
B-ALP at 12 months



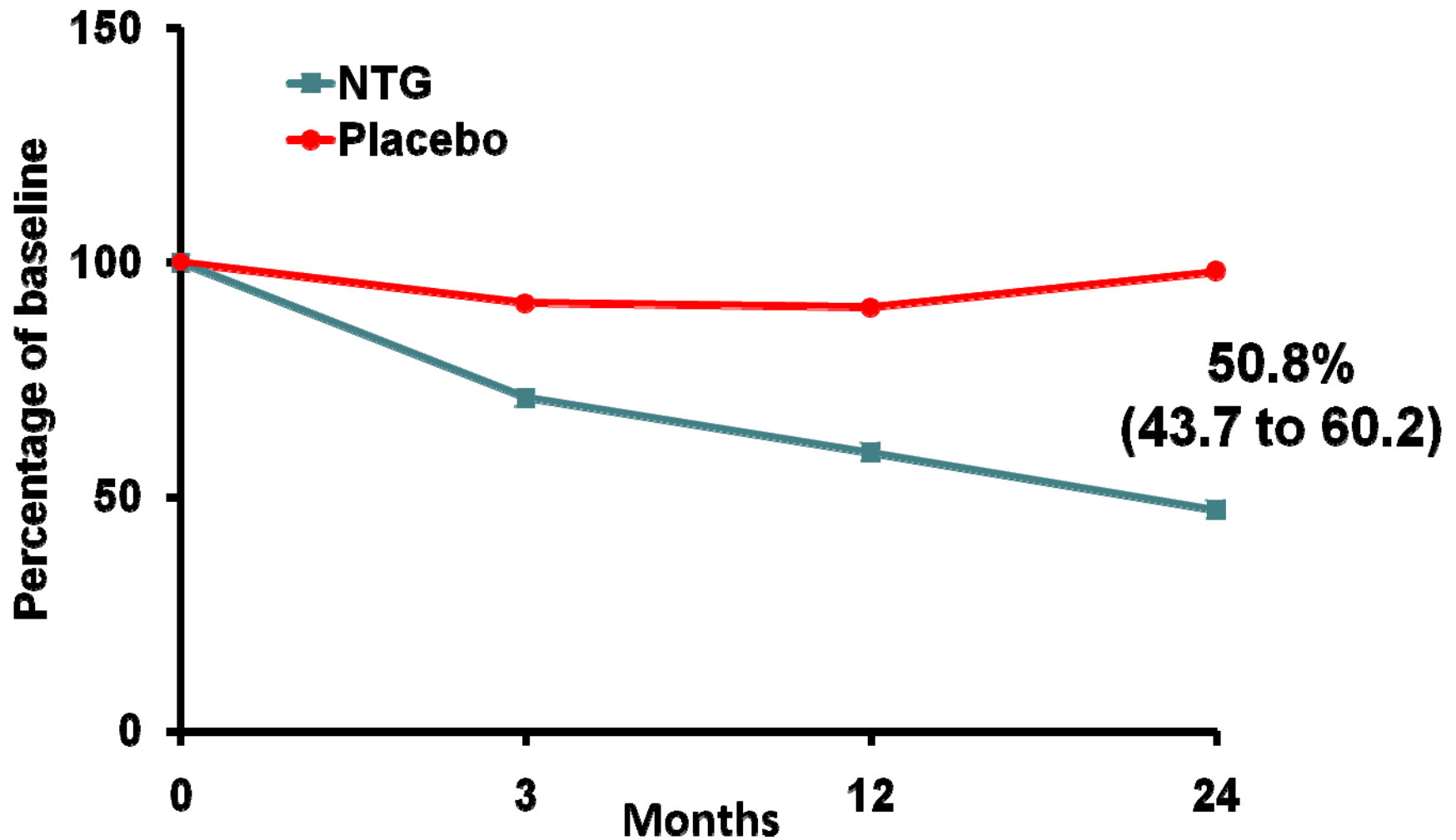
B-ALP at 24 months



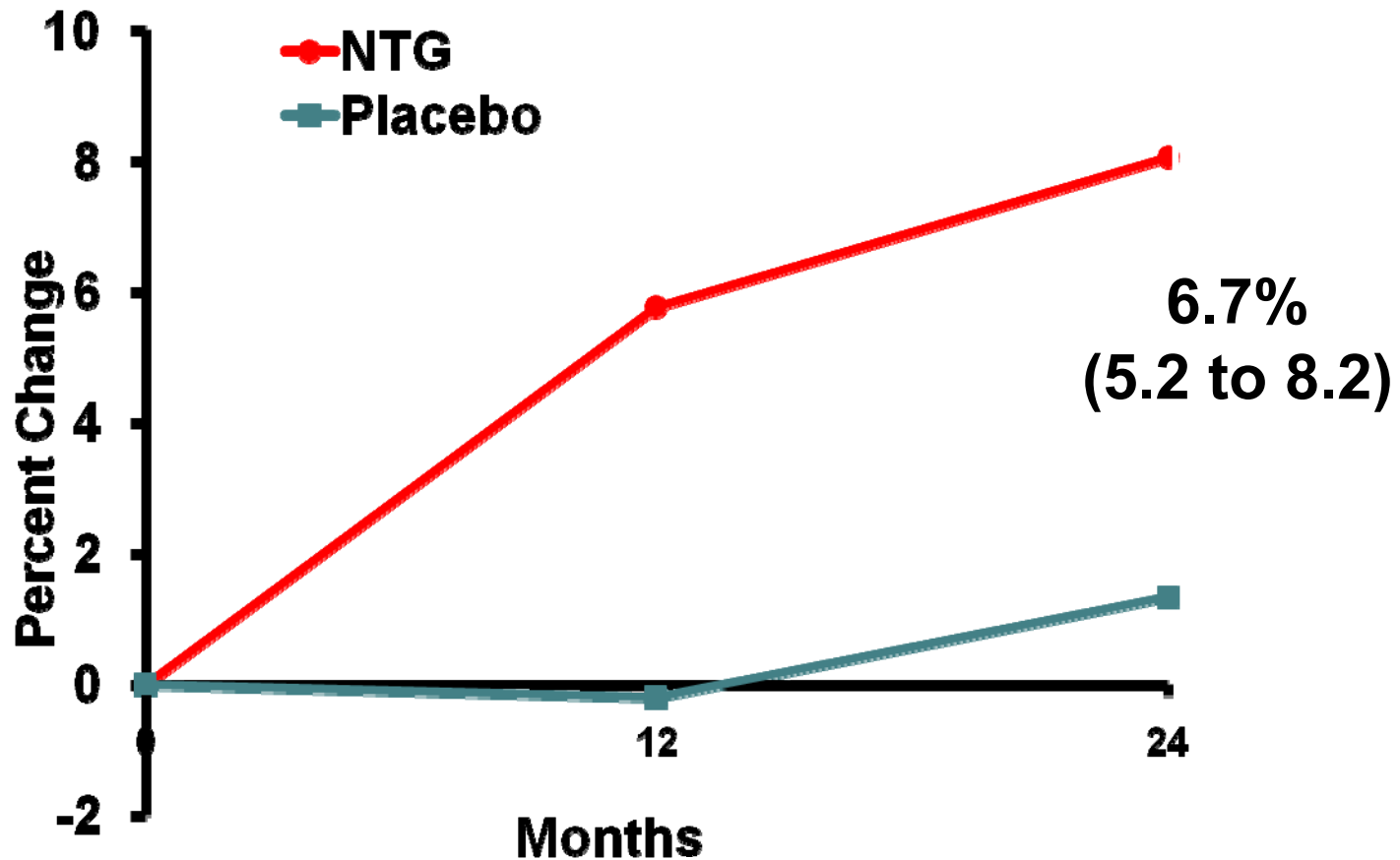
NTX at 12 months



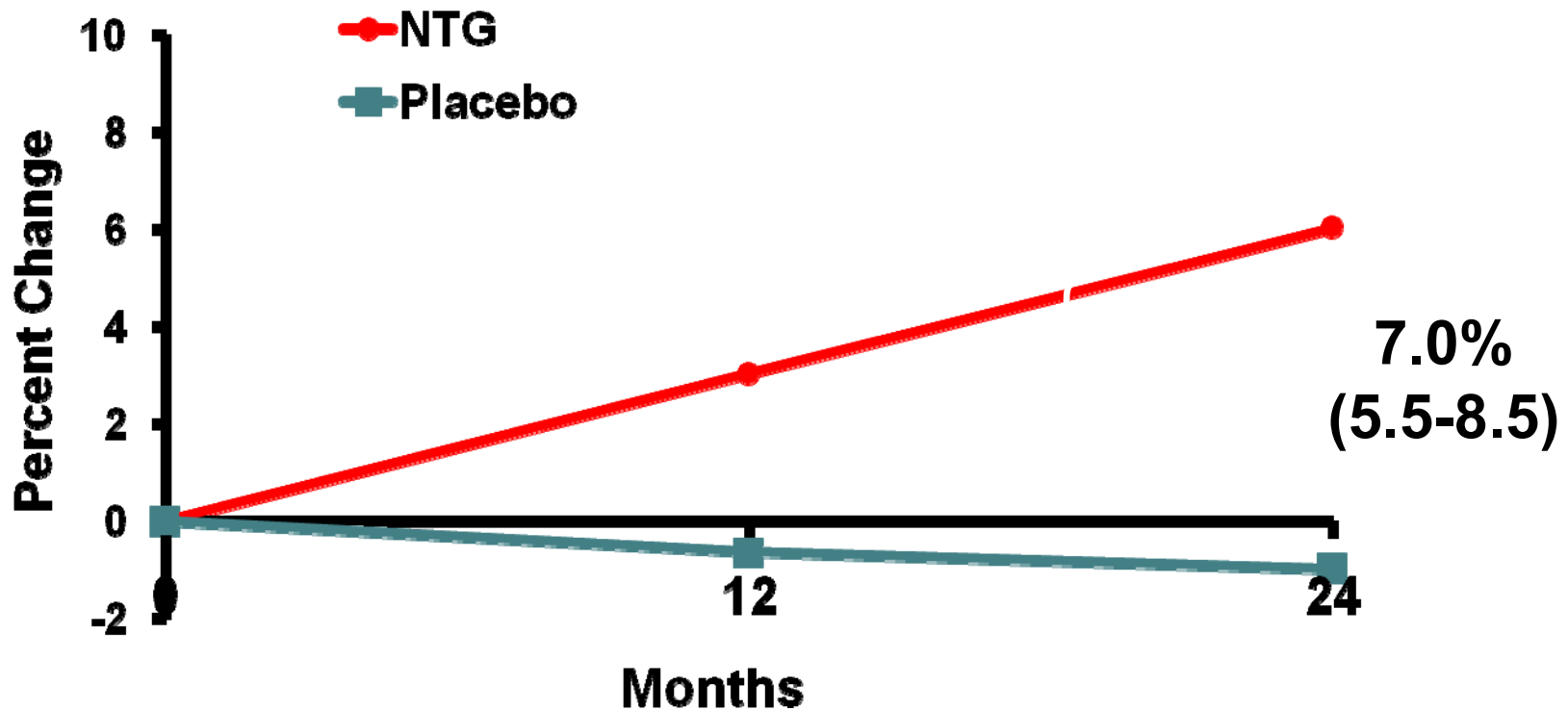
NTX at 24 months



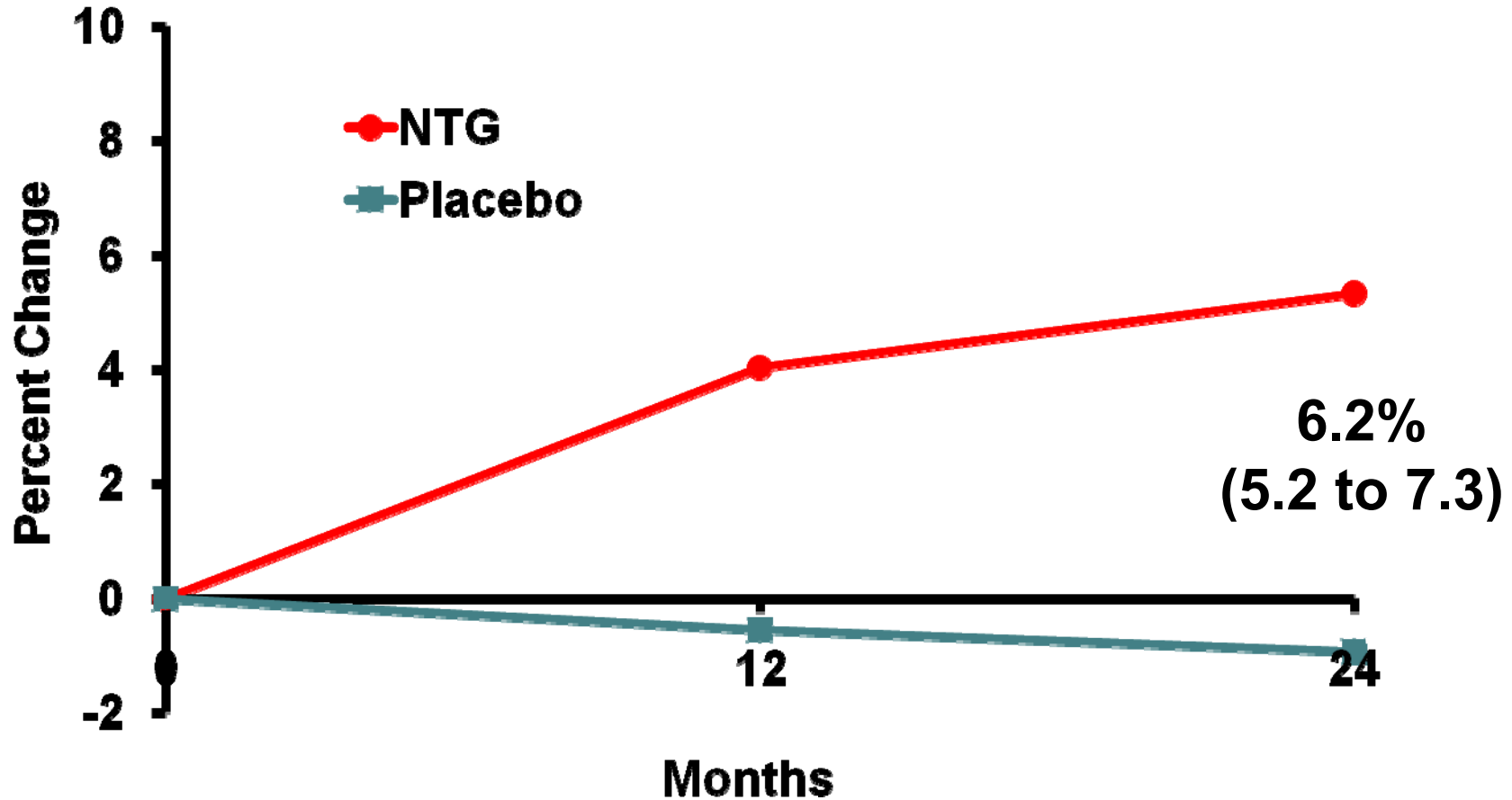
Lumbar Spine BMD at 24 months



Femoral Neck BMD at 24 months



Total Hip BMD at 24 months





PQCT of the Radius

Measurement	% Difference (95%CI)
Trabecular density	11.9 (8.1 to 15.7)
Cortical thickness	13.9 (6.0 to 21.7)
Cortical density	2.2 (0.6 to 3.7)
Cortical area	10.6 (6.9 to 14.3)
Periosteal circumference	7.4 (4.3 to 10.4)



Indices of Radial Bone Strength

Measurement	% difference (95%CI)
Polar Section Modulus	10.7 (7.5 to 13.8)
Polar Moment of Inertia	7.3 (4.6 to 10.1)



PQCT of the Tibia

Measurement	% Difference (95%CI)
Trabecular density	8.5 (4.3 to 12.7)
Cortical thickness	24.6 (18.9 to 30.4)
Cortical density	1.5 (0.8 to 2.3)
Cortical area	10.0 (5.2 to 15.0)
Periosteal circumference	2.9 (1.0 to 6.8)



Indices of Tibial Bone Strength

Measurement	% difference (95%CI)
-------------	----------------------

Polar Section Modulus	9.8 (0.2 to 19.4)
-----------------------	-------------------

Polar Moment of Inertia	14.5 (3.2 to 25.8)
-------------------------	--------------------



Headaches

Run In Phase 400 enrolled, 104 did not continue

Treatment	Nitroglycerin	Placebo
d/c at 12 months	7 (23.3%)	2 (13.3%)
1 month	35%	5.4%
12 months	5.3%	0.9%
24 months	2%	0



	Nitroglycerin	Placebo
Serious Adverse events	5 (4.2%)	5 (4.3%)
-Death	1 (1%)	0
Fractures	2 (1.6%)	2 (1.7%)



Conclusions

- Nitroglycerin uncouples bone turnover
- Improves BMD, geometry, indices of strength
- Inexpensive and widely available
- Initiation of treatment limited by headache
- The efficacy of nitroglycerin to reduce fracture risk should be tested in a clinical trial