

Continuous infusion of PGE₂ is catabolic with a negative bone balance on both cancellous and cortical bone in rats

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Abstract

It is well documented that intermittent PGE₂ treatment increases both trabecular and cortical bone mass. However, the effects of continuous PGE₂ administration remain undocumented. The aim of the study was to investigate the effects of continuous prostaglandin E₂ (PGE₂) on different bone sites in skeletally mature rats. Six-month-old Sprague Dawley rats were treated with PGE₂ at 1 or 3 mg/kg/d continuously via infusion pump for 21 days. Two other groups of rats received PGE₂ at the same doses by intermittent (daily) subcutaneous injections and served as positive controls. Histomorphometry was performed on cancellous bone of the proximal tibial metaphysis and cortical bone of the tibial shaft. As expected, intermittent PGE₂ treatment increased both cancellous and cortical bone mass by stimulating bone formation at the cancellous, periosteal and endocortical bone surfaces. In contrast, continuous PGE₂ treatment decreased cancellous bone mass with bone resorption exceeding bone formation. In addition, continuous PGE₂ treatment increased endocortical and intracortical bone remodeling, inducing bone loss which was partially offset by stimulating periosteal expansion. We conclude that continuous PGE₂ treatment induces overall catabolic effects on both cancellous and cortical bone envelopes, which differs from intermittent PGE₂ treatment that is anabolic. Lastly, we speculate that superior bone mass may be achieved by co-treatment of continuous PGE₂ in combination with an anti-catabolic agent.

Keywords: Prostaglandin E₂, Bone Formation, Bone Resorption, Bone Balance

Introduction

The skeletal anabolic effect of intermittently administered Prostaglandin E₂ (PGE₂) has been demonstrated in intact rats of various ages, gonadectomized male and female rats, and in dogs and infants¹⁻¹⁵. It was assumed that similar to continuous PTH administration, continuous administration of PGE₂ would be catabolic¹⁶. Long-term continuously administered PGE₂ in infants with cyanotic congenital heart disease resulted in cortical hyperostosis of long bones due to periosteal bone formation, which was reversed upon with-

drawal of PGE₂¹⁷. The only continuous PGE₂ treatment study was performed by Desimone et al. in 7-week-old female rats, where he found that PGE₂ continuous administration by subcutaneous implantation of a controlled pellet caused cancellous bone loss with no periosteal or endosteal bone formation responses¹⁸. This lack of periosteal bone formation differed from the findings by Ueda et al. in infants¹⁷⁻²⁰. However, in Desimone's model, it was believed that PGE₂ induced inflammation responses that were responsible for the loss in cancellous bone. The authors speculated that the pellet administration route might not be a reliable method of delivering PGE₂ systemically. As there is abundant evidence that daily PGE₂ administration is a reliable bone growing treatment regimen, we believe there is a need to clarify if continuous PGE₂ treatment could achieve the same anabolic effects as daily PGE₂ in adult female rat skeletons.

This experiment was designed to evaluate the effects of continuous PGE₂ treatment on cancellous and cortical bone histomorphometry profiles in 6-month-old female rats as compared to those with intermittent PGE₂ treatment. Histomorphometry of the proximal tibial metaphysis (PTM)

Authors Tian, Zhang, Zhao, Setterberg and Jee have no conflict of interest. Authors Zeng and Ma have corporate appointments with Eli Lilly Company.

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Groups	%Tb.Ar (%)	Tb.Wi (μm)	Tb.N (#/mm)	Tb.Sp (μm)	%O.Pm (%)	%L.Pm (%)	MAR (μm/d)	BFR/BS (μm ³ /μm ² /d)	%Er.Pm (%)
Baseline	21.08±5.19	50.63±4.78	4.14±0.83	203.50±74.41	9.09±2.23	34.92±5.08	0.89±0.03	31.10±4.56	0.95±0.26
Control	22.68±3.37	49.85±2.58	4.57±0.77	175.13±40.15	10.93±1.94	34.96±2.89	0.87±0.03	30.45±2.61	0.87±0.20
Continuous 1 mg	13.48±2.83 ^a (-41)	39.29±3.06 ^a (-20)	3.36±0.54 ^a (-26)	264.03±51.34 ^a (51)	17.51±2.38 ^a (60)	33.53±3.16 (-4)	0.88±0.03 (1)	29.66±3.30 (-3)	1.77±0.07 ^a (102)
Continuous 3 mg	11.03±1.86 ^a (-51)	41.75±3.83 ^a (-16)	2.63±0.23 ^{ab} (-42)	341.17±35.89 ^{ab} (95)	26.82±5.94 ^{ab} (145)	44.56±3.27 ^{ab} (27)	0.97±0.04 ^{ab} (11)	43.27±3.33 ^{ab} (42)	2.68±0.14 ^{ab} (207)
Intermittent 1 mg	26.52±2.95 ^{abc} (17)	59.32±5.42 ^{abc} (19)	4.47±0.21 ^{bc} (-2)	165.03±13.06 ^{bc} (-6)	14.41±2.85 ^{ac} (32)	45.53±3.28 ^{ab} (30)	0.92±0.03 ^{ac} (5)	41.76±3.88 ^{ab} (37)	1.05±0.12 ^{bc} (20)
Intermittent 3 mg	25.86±4.50 ^{bc} (14)	63.70±7.43 ^{abc} (28)	4.06±0.51 ^{bc} (-11)	185.88±30.83 ^{bc} (6)	18.02±1.82 ^{acd} (65)	50.36±2.35 ^{abc,d} (44)	0.98±0.03 ^{abd} (12)	49.06±1.52 ^{abc,d} (61)	1.23±0.23 ^{abc} (41)

Mean±SD (% change from Control). Note: a: vs. Control; b: vs. Continuous 1 mg; c: vs. Continuous 3 mg; d: vs. Intermittent 1 mg; *p*<0.05.

Table 1. Static and dynamic histomorphometric changes in proximal tibial metaphysis.

Groups	BFR/BV (%/yr)	W.Wi (μm)	FP (d)	RP (d)	Rm.P (d)	Act.F (cycle/yr)	BFR/BS/%Er.Pm	%O.Pm/%Er.Pm
Baseline	377.25±66.85	12.89±1.06	14.47±1.13	1.56±0.50	16.02±1.18	2.31±0.61	35.30±12.24	10.11±3.06
Control	373.26±41.98	13.40±0.92	15.38±0.88	1.24±0.30	16.62±0.97	2.59±0.42	36.50±8.49	12.85±2.32
Continuous 1 mg	454.79±62.22 ^a (22)	12.08±0.59 ^a (-10)	13.70±0.87 ^a (-11)	1.40±0.21 (13)	15.10±0.99 ^a (-9)	4.69±0.79 ^a (81)	16.74±1.57 ^a (-54)	9.90±1.28 ^a (-23)
Continuous 3 mg	634.20±60.99 ^{ab} (70)	12.19±0.54 ^a (-9)	13.40±0.92 ^a (-13)	1.41±0.37 (13)	14.80±1.17 ^a (-11)	7.37±1.81 ^{ab} (184)	16.13±1.06 ^a (-56)	9.97±2.00 ^a (-22)
Intermittent 1 mg	430.94±48.21 ^{ac} (15)	14.98±0.69 ^{abc} (12)	16.36±0.90 ^{bc} (6)	1.24±0.34 (0)	17.60±1.05 ^{bc} (6)	3.22±0.65 ^{bc} (24)	40.40±7.44 (11)	13.94±3.39 (8)
Intermittent 3 mg	474.16±54.31 ^{ac} (27)	14.25±0.80 ^{abc} (6)	14.63±1.01 ^{cd} (-5)	1.00±0.18 ^{bc} (-19)	15.63±1.10 ^d (-6)	4.51±0.52 ^{acd} (74)	41.63±11.81 (14)	15.03±2.87 (17)

Mean±SD (% change from Control). Note: a: vs. Control; b: vs. Continuous 1 mg; c: vs. Continuous 3 mg; d: vs. Intermittent 1 mg; *p*<0.05.

Table 2. Bone remodeling and bone balance changes in proximal tibial metaphysis.

and the tibial shaft (TX) sites was determined. In addition, we propose co-treatment with continuous PGE₂ in combination with an anti-catabolic agent might generate a greater bone gain and bone strength than intermittent administration of PGE₂.

Materials and methods

Forty-eight, six-month-old, virgin female Sprague Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, IN), weighing approximately 220 g, were maintained on a 12-hour light/12-hour dark cycle at 22°C with *ad libitum* access to food (TD 5001 with 0.95% calcium and 0.67% phosphorus, vitamin D₃ 4500 IU/kg; Teklad, Madison, WI) and water. Eight rats were euthanized as the baseline controls, while the remaining rats were randomly divided into 5 groups with

eight rats in each group. Prostaglandin E₂ (Upjohn Company, Kalamazoo, MI) at 1 or 3 mg/kg/d was given to the rats by continuous infusion via Infu-Disk™ pump (Med-e-Cell, San Diego, CA) or daily subcutaneous (sc) injections on the back for 21 days. The Infu-Disk™ pumps were connected to the jugular vein and placed on the back with a special jacket, and changed every 7 days. Prostaglandin E₂ was first dissolved in ethanol then further diluted into the final injection solution (10% ethanol with 1 mL/kg injection volume). All rats received sc injections with Calcein (5 mg/kg; Sigma, St Louis, MO) on days 10, 9, 3 and 2 before sacrifice. The animal protocol was approved by the Institutional Animal Care and Use Committee of Eli Lilly Research Laboratories to ensure compliance with NIH guidelines.

At necropsy, the right tibia was removed, cleaned of soft tissue, and fixed in 70% ethanol. Bones were first stained for

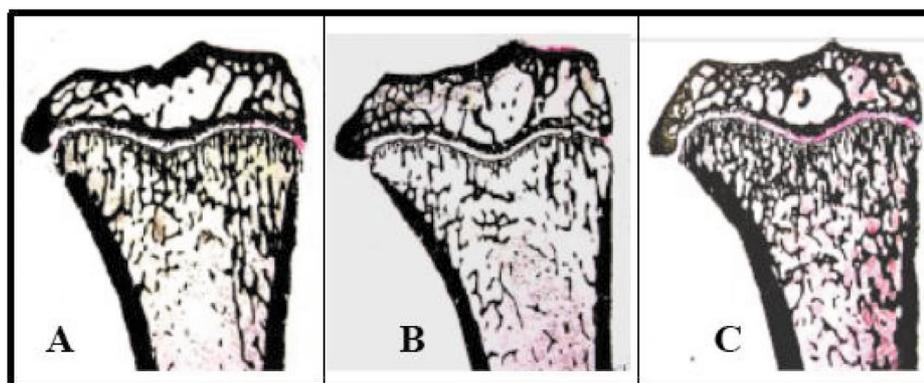


Figure 1. Von Kossa stained sections of proximal tibial metaphysis from (A) control, (B) continuous 3 mg PGE₂/kg/d and (C) intermittent 3 mg PGE₂/kg/d show reduced trabecular bone mass with continuous treatment and increased trabecular bone mass with intermittent treatment compared to controls.

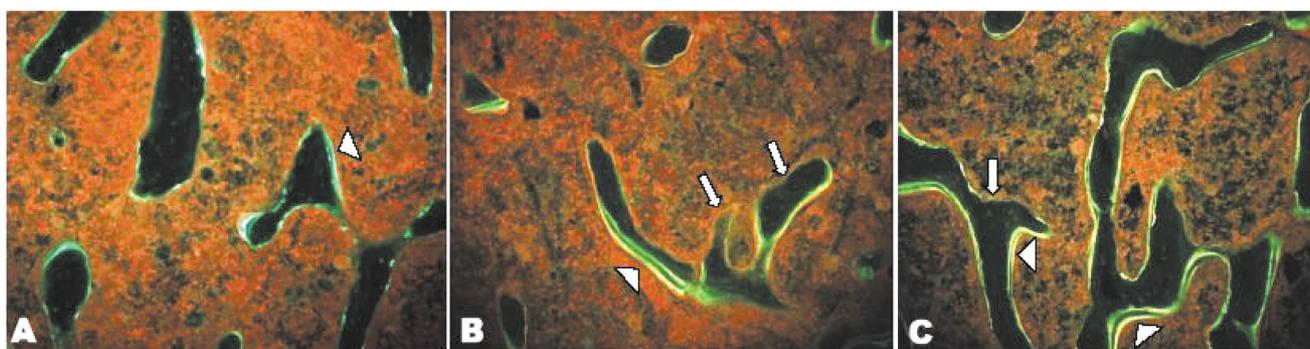


Figure 2. Villanueva bone stained sections of proximal tibial metaphysis under fluorescent microscope from (A) control, (B) continuous 3 mg PGE₂/kg/d and (C) intermittent 3 mg PGE₂/kg/d administration showing morphology and fluorochrome labeling. Arrows denote eroded surfaces, arrowheads denote double fluorescent labeling surface. Continuous (B) treatment resulted in lower and thinner trabeculae and increased eroded perimeter greater than labeled perimeter; and intermittent (C) administration caused increased and thicker trabeculation, and abundant double-labeled surfaces with formation greater than resorption. 10X magnification.

4 days in Villanueva osteochrome bone stain (Arizona Histology & Histomorphometry Center, Phoenix, AZ, USA), then dehydrated in a graded ethanol series, defatted in acetone, and embedded in methyl methacrylate. Longitudinal sections of 20 μ m thickness PTM or cross sections of 30 mm thickness tibial-fibular junction were prepared for analysis. Measurements for cancellous bone were performed on the entire marrow region within the cortical shell of PTM between 1 and 4 mm distal to the growth plate-metaphyseal junction using an Image Analysis System (Osteomeasure, Inc., Atlanta, GA). Bone area, perimeter, single- and double-labeling surfaces, and eroded surface on trabecular or cortical bones were measured. Trabecular number, thickness, mineral appositional rate, bone formation rate–surface reference (BFR/BS), bone volume (BFR/BV) and activation frequency were calculated as described previously^{21,22}. For the cortical bone of the TX, static and dynamic parameters were

measured and calculated according to Jee et al.²³.

Data are presented as mean \pm SD. Statistics were calculated by an Ultimate Integrated Data Analysis and Presentation System (StatView 5.0.1, SAS Institute Inc., Cary, NC, USA). Across group comparisons were made with ANOVA followed by Fisher's protected least significant difference test (PLSD). Differences were considered statistically significant at $p < 0.05$ on a two-tailed test.

Results

Continuous PGE₂ treatment in cancellous bone of the proximal tibial metaphysis (PTM) compared to aging control (Tables 1, 2; Figures 1B, 2B)

Continuous infusion of 1 and 3 mg/kg/d for 21 days decreased trabecular bone area (%Tb.Ar: -41% and -51%),

Group	T.Ar mm ²	Ma.Ar mm ²	CtB.Ar mm ²	%CtB.Ar %	Ct.Th mm	Ic-%Po.Ar %	Ec-Wo.B frequency	Ec-BFR/ Ec-%Er.Pm
Baseline	4.93±0.19	0.82±0.08	4.10±0.15	82.76±1.14	703.36±14.02	0.43±0.23		10.24±7.97
Control	4.91±0.16	0.87±0.11	4.01±0.14	81.44±2.05	686.09±24.34	0.47±0.16		7.25±1.02
Continuous 1 mg	4.97±0.35 (1)	0.86±0.14 (-2)	4.07±0.34 (1)	81.11±2.85 (0)	700.97±50.28 (2)	0.98±0.27 ^a (111)		4.10±2.30 ^a (-43)
Continuous 3 mg	5.03±0.41 (2)	0.86±0.08 (-2)	4.09±0.39 (2)	79.77±1.97 ^a (-2)	721.49±43.33 (5)	1.85±0.52 ^{a,b} (298)		5.19±1.73 ^a (-28)
Intermittent 1 mg	4.62±0.24 ^{b,c} (-6)	0.69±0.06 ^{a,b,c} (-21)	3.92±0.19 (-2)	84.40±0.53 ^{a,b,c} (4)	701.19±21.10 (2)	0.44±0.12 ^{b,c} (-4)	3/8	18.09±12.93 ^{a,b,c} (150)
Intermittent 3 mg	4.72±0.38 ^e (-4)	0.72±0.10 ^{a,b,c} (-17)	4.00±0.42 (0)	83.68±2.40 ^{a,b,c} (3)	707.35±52.08 (3)	0.53±0.18 ^{b,c} (12)	4/8	22.48±17.89 ^{a,b,c} (254)

Mean±SD (% change from Control). Note: a: vs. Control; b: vs. Continuous 1 mg; c: vs. Continuous 3 mg; d: vs. Intermittent 1 mg; p<0.05. Ic-: intracortical; Ec-: endocortical surface.

Table 3. Static and dynamic histomorphometric changes in tibial shaft.

Group	Ps-%L.Pm %	Ps-MAR µm/d	Ps-BFR µm/d*100	NL-B.Ar mm ²	Wo-B.Ar mm ²	Ec-%L.Pm %	Ec-MAR µm/d	Ec-BFR µm/d*100	Ec-%Er.Pm %
Baseline	42.78±17.08	0.75±0.35	36.40±25.24			13.64±4.33	0.57±0.25	8.24±5.08	0.90±0.65
Control	42.13±14.38	0.94±0.12	39.31±14.06			10.38±4.07	0.51±0.12	5.36±2.26	0.56±0.32
Continuous 1 mg	84.92±9.94 ^a (102)	2.03±0.28 ^a (116)	172.89±36.04 ^a (340)	0.23±0.07		25.07±4.38 ^a (142)	0.58±0.12 (12)	14.83±5.23 ^a (177)	3.52±1.86 ^a (524)
Continuous 3 mg	85.28±10.67 ^a (102)	2.47±0.37 ^a (164)	210.65±37.48 ^{a,b} (436)	0.19±0.04	0.42±0.23	33.99±5.92 ^a (227)	0.95±0.38 ^{a,b} (84)	33.40±18.98 ^{a,b} (523)	5.06±1.68 ^{a,b} (798)
Intermittent 1 mg	65.51±15.21 ^{a,b,c} (56)	1.20±0.11 ^{a,b,c} (28)	79.58±22.50 ^{a,b,c} (102)	0.04±0.02		17.40±8.18 ^a (68)	0.96±0.16 ^{a,b} (87)	17.52±9.78 ^{a,c} (227)	1.19±0.55 ^{b,c} (112)
Intermittent 3 mg	72.37±13.19 ^a (72)	1.44±0.24 ^{a,b,c} (53)	105.90±33.50 ^{a,b,c} (169)	0.07±0.03		20.85±9.97 ^a (101)	0.90±0.31 ^{a,b} (76)	20.73±8.91 ^a (287)	1.05±0.40 ^{b,c} (87)

Mean±SD (% change from Control). Note: a: vs. Control; b: vs. Continuous 1mg; c: vs. Continuous 3mg; d: vs. Intermittent 1mg; p<0.05. Ps-: periosteal surface; NL: new lamellar bone; Wo: woven bone; Ec-: endocortical surface.

Table 4. Periosteal and endocortical surface histomorphometric changes in tibial shaft.

trabecular width (Tb.Wi: -20% and -16%) and trabecular number (Tb.N: -26% and -42%), but increased trabecular separation (Tb.Sp: +51% and +95%). It markedly stimulated bone turnover (BFR/BV: +22% and +70%) and activation frequency (Act.F: +81% and +184%), shortened the remodeling period (Rm.P: -9% and -11%), and decreased the formation period (FP: -11% and -13%). Bone eroded parameter was markedly elevated (%Er.Pm: +102% and +207%), while the increase in bone formation was only significant at the 3 mg/kg/d dose level (BFR/BS: +42%). Wall width was decreased for both PGE₂ doses we studied (W.Wi: -10% and -9%). The estimated cancellous bone balance (BFR/BS/%Er.Pm: -54% and -56%, and %O.Pm/%Er.Pm: -23% and -22%) were decreased compared to controls for both doses.

Continuous PGE₂ treatment in cortical of the tibial shaft (TX) compared to aging control (Tables 3, 4; Figure 3B)

Continuous treatment of 1 and 3 mg/kg/d for 21 days resulted in no significant changes in static parameters. Periosteal bone formation (Ps-BFR: +340% and +436%) was increased. At the endocortical surface, there were negative endocortical bone balances for both PGE₂ doses (Ec-BFR/Ec-%Er.Pm: -43% and -28%) with bone resorption (Ec-%Er.Pm: +5.24 and +7.98 times) increased to larger degrees than bone formation (Ec-BFR: 1.77 and 5.23 times). Intracortical porosity areas were increased dramatically (Ic-%Po.Ar: +111% and +298%). The two doses laid down new subperiosteal lamellar bone (NL-B.Ar: 0.23 + 0.07 mm² and 0.19 + 0.04 mm²). In addition, the 3mg/kg dose formed new subperiosteal woven bone (Wo-B.Ar: 0.42 + 0.23 mm²).

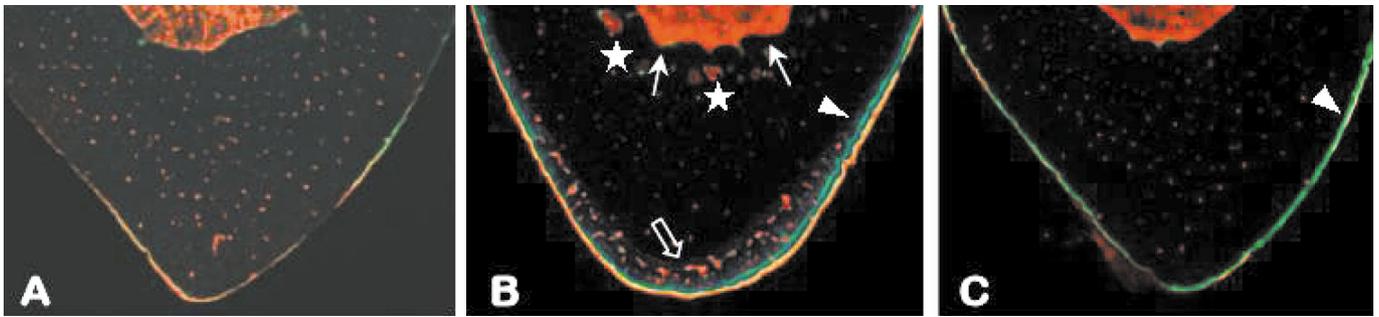


Figure 3. Villanueva bone stained sections of tibial shaft under fluorescent microscope from (A) control, (B) continuous 3 mg PGE₂/kg/d and (C) intermittent 3 mg PGE₂/kg/d administration showing increased endocortical eroded surface (arrows), intracortical porosity adjacent to marrow (stars), stimulated subperiosteal bone formation (open arrow) and double-labeled periosteal surfaces (arrowhead) after continuous treatment. Intermittent treatment was limited mainly to increased double-labeled periosteal surfaces (arrowhead).

Intermittent PGE₂ treatment in cancellous bone of the proximal tibial metaphysis (PTM) compared to aging controls (Tables 1, 2; Figures 1C, 2C).

The 1 and 3 mg/kg/d by intermittent treatment (daily, subcutaneous injection) increased trabecular bone mass (%Tb.Ar: +17% and +14% ns) and width (Tb.Wi: +19% and +28%). It stimulated bone turnover (BFR/BV: +15% and +27%) and remodeling as reflected by activation frequency (Act.F: +24% and +74%), which were associated with an imbalance in bone resorption (%Er.Pm: +41% with the 3 mg dose) and bone formation in favor of bone formation (BFR/BS: +37% and +61%) that led to an estimated positive cancellous bone balance (BFR/BS/%Er.Pm: +11% and +14%) and increased wall width (W.Wi: +12% and +6%).

Intermittent PGE₂ treatment in cortical bone of the tibial shaft of (TX) compared to aging control (Tables 3, 4; Figure 3C).

Intermittent treatment of 1 and 3 mg PGE₂ increased cortical bone mass (%CtB.Ar: +4% and +3%) and decreased marrow cavity size (Ma.Ar: -21% and -17%) from a combination of stimulated periosteal bone formation (Ps-BFR: +102% and +169%) and a positive endocortical bone balance (Ec-BFR/Ec-%Er.Pm: +150% and +254%). Three and four out of eight rats exhibited marrow trabeculae arising from the endocortical surface with the 1 and 3 mg/kg doses, respectively (Ec-Wo.B frequency: 3/8 and 4/8).

Comparisons of continuous and intermittent PGE₂ administration in cancellous bone (PTM) by continuous to intermittent ratios (C/I ratios).

Table 5 shows numerous opposing responses from the catabolic effects of continuous and anabolic effects of intermittent treatment. These include trabecular bone mass, architecture, wall width, bone balance, bone formation rate and formation period. At the 3 mg/kg/d dose level, continuous to intermittent

(C/I) ratios in favor of continuous treatment involve index of bone resorption (%Er.Pm: 5.1), bone turnover (BFR/BV: 2.6), bone remodeling (Act.F: 2.5), and osteoid perimeter (%O.Pm: 2.2). A continuous to intermittent ratio in favor of intermittent administration was the bone formation rate (BFR/BS: 0.7).

Comparisons of continuous and intermittent PGE₂ administration in cortical bone (TX) by continuous to intermittent ratios (C/I ratios).

Table 6 shows skeletal responses to cortical bone. At the 3 mg/kg/d dose level, the opposing responses to the treatments were limited to cortical bone area (%CtB.Ar: -2% (ns) vs. +3%), and a negative endocortical bone balance for continuous and a positive for intermittent delivery (Ec-BFR/Ec-%Er.Pm: -28% vs. +254%). The major differences between the two treatment routes were that continuous treatment was more efficacious in inducing positive C/I ratios for endocortical bone resorption (Ec-%Er.Pm: 9.2), bone formation (Ec-BFR: 1.8), and a striking effect in intracortical remodeling (Ic-%Po.Ar: 24.8).

Discussion

Continuous PGE₂ treatment elicited a catabolic effect on the cancellous bone compartment of the PTM. The treatment stimulated bone turnover, remodeling, resorption, and formation with a negative cancellous bone balance that reduced bone mass and deteriorated bone architecture. It also induced a negative bone balance in the cortical bone as it increased endocortical and intracortical bone remodeling which offset the bone gain from the periosteal surface.

Previous studies using continuous PGE₂ administration were limited to a human study on infants where Ueda et al.¹⁷ reported continuous PGE₂ in infants resulted in cortical hyperostosis of long bones with higher periosteal bone formation¹⁷⁻²⁰ which was reversed upon withdrawal of PGE₂¹⁷. It is believed that the

Parameters	Abbreviations	Continuous 1 mg (% change from Control)	Intermittent 1 mg (% change from Control)	C/I	Continuous 3 mg (% change from Control)	Intermittent 3 mg (% change from Control)	C/I
Percentage trabecular bone mass	%Tb.Ar	-41	17	-	-51	14 (ns)	-
Trabecular width	Tb.Wi	-20	19	-	-16	28	-
Wall width	W.Wi	-10	12	-	-9	6	-
Percentage osteoid perimeter	%O.Pm	60	32	1.9	145	65	2.2
Percentage trabecular eroded perimeter	%Er.Pm	102	20 (ns)	5.1	207	41	5.1
Bone formation rate/bone volume	BFR/BV	22	15	1.5	70	27	2.6
Bone formation rate/bone perimeter	BFR/BS	-3 (ns)	37	-	42	61	0.7
Activation frequency	Act.F	81	24	3.4	184	74	2.5

Note: ns: non-significant changes; -: opposing response.

Table 5. Continuous and intermittent (C/I) PGE₂ effectiveness ratios in proximal tibial metaphysis from selected histomorphometric parameters.

Parameters	Abbreviation	Continuous 1 mg (% change from Control)	Intermittent 1 mg (% change from Control)	C/I	Continuous 3 mg (% change from Control)	Intermittent 3 mg (% change from Control)	C/I
Percentage cortical bone area	%CtB.Ar	0	4	0	-2 (ns)	3	-
Periosteal bone formation rate	Ps-BFR	340	102	3.3	436	169	2.6
Endocortical bone formation rate	Ec-BFR	177	227	0.8	523	287	1.8
Endocortical percentage eroded perimeter	Ec-%Er.Pm	524	112 (ns)	4.7	798	87 (ns)	9.2
Percentage intracortical porosity area	Ic-%Po.Ar	111	-4 (ns)	-	298	12 (ns)	24.8
Endocortical bone formation rate to percentage eroded perimeter ratio	Ec-BFR / Ec-%Er.Pm	-43	150	-	-28	254	-

Note: ns: non-significant changes; -: opposing response.

Table 6. Continuous and intermittent (C/I) PGE₂ effectiveness ratios in cortical bone of tibial shaft from selected histomorphometric parameters.

cortical hyperostosis was formed of woven bone that could be resorbed on cessation of treatment^{5,24}. An animal study that was done by Desimone et al. used rapidly growing rats that were implanted with PGE₂ loaded pellets where he found PGE₂ caused cancellous bone loss with no periosteal bone formation¹⁸. These authors concluded the skeletal responses in their models were inflammatory responses rather than the effects of continuous PGE₂ administration. Thus, the current study is the first to describe the skeletal effects of the continuous PGE₂ administration, which was similar to continuous PTH administration that caused cancellous bone loss²⁵⁻²⁷. However, in the current study, we found that continuous PGE₂ administration induced the largest bone gain at the periosteal bone surface, which was superior to intermittent PGE₂ treatment^{3,5,6,9,11,15,28,29}.

Our current study confirmed that intermittent PGE₂

administration induced a mild anabolic response that increased trabecular bone mass and thickness. The response mimicked that of the classical anabolic agent¹⁶. It stimulated bone turnover and remodeling coupled to a positive cancellous bone balance – an imbalance of bone resorption and formation in favor of bone formation^{4,8}. In cortical bone, there was an anabolic response at both the periosteal and endocortical bone surfaces, which increased cortical bone mass and decreased marrow cavity size. Direct stimulation of periosteal and marrow trabecular bone formation occurred, resulting in positive endocortical bone balance. These findings are in agreement with previous studies^{3,5,6,11,24,28,29}.

We were able to make direct comparisons of some key parameters of bone architectural and bone remodeling following continuous and intermittent treatments by using continu-

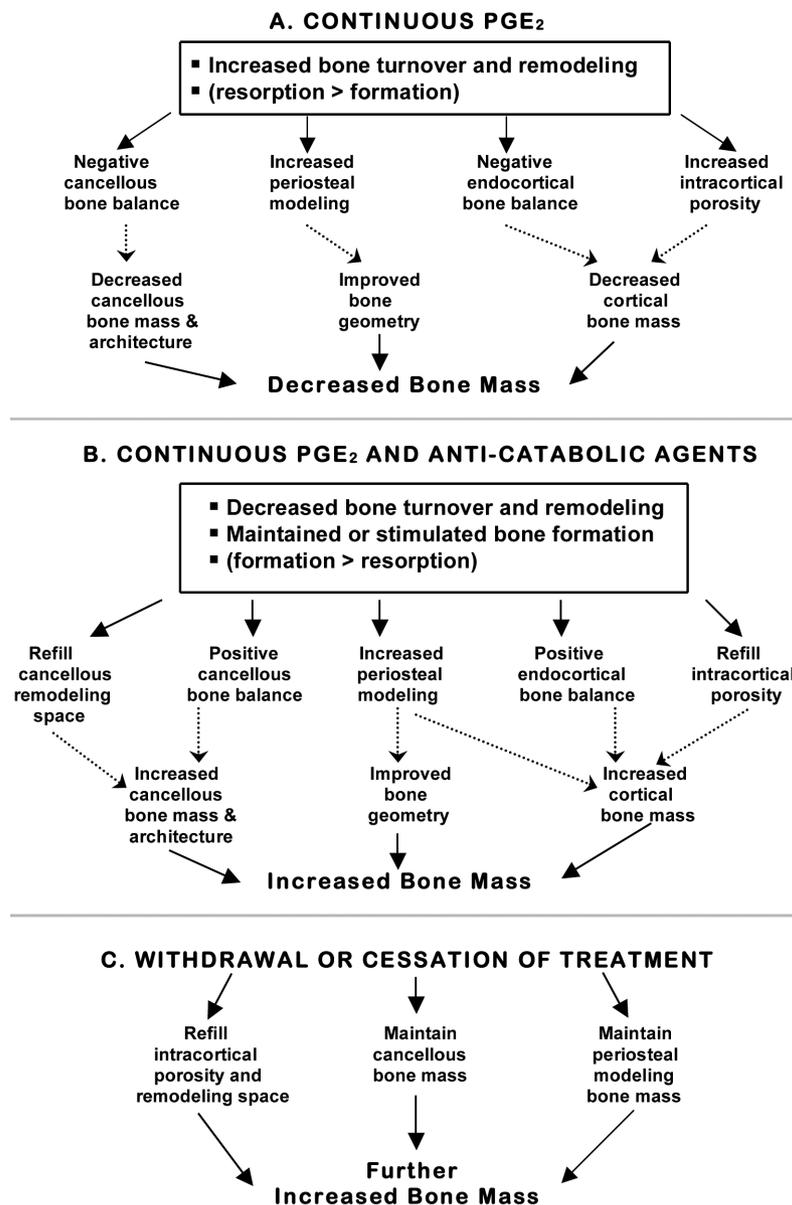


Figure 4. Flow diagram of mechanisms of decreased bone strength after continuous PGE₂ administration (A), increased bone strength from co-treatment with continuous PGE₂ and an anti-catabolic agent [estrogen, bisphosphonates, selective estrogen receptor modulator and osteoprotegerin] (B) and further improvement in bone strength upon cessation of treatment (C). See text for more details.

ous to intermittent treatment (C/I) ratios. When compared to intermittent treatment, continuous administration was shown to be a stronger activator of bone turnover and remodeling (2.5-fold) with an increased index of bone resorption (5.1-fold). However, continuous administration yielded 30% less stimulation of cancellous bone formation versus intermittent treatment (Table 5). In cortical bone, continuous treatment was found to be more powerful in periosteal bone formation (2.6-fold), endocortical bone resorption (9.2-fold), endocortical bone formation (1.8-fold), and intracortical porosity (24.8-fold) compared to intermittent administration (Table 6).

It is generally accepted that stimulated periosteal bone formation to expand the total cortical circumference is an effective mechanism to dramatically improve bone strength and reduce fracture risk³⁰⁻³⁴. The present findings show continuous administration of PGE₂ is a powerful way to stimulate periosteal bone formation, which could improve bone strength. The 1 and 3 mg/kg/d doses of continuous PGE₂ elevated periosteal bone formation 3.4- and 4.4-fold, respectively, as compared to aging controls. In contrast, intermittent delivery stimulated periosteal formation to a lesser degree, 102% and 169%, respectively. The continuous route proved to be 2.6-fold more powerful than the

intermittent one (Tables 4 and 5). The 1 mg/kg/d of continuous PGE₂ formed more lamellar bone ($0.23 \pm 0.07 \text{ mm}^2$) compared to 1 mg/kg/d intermittent ($0.04 \pm 0.02 \text{ mm}^2$). Likewise, the 3 mg/kg/d continuous dose formed more lamellar bone ($0.19 \pm 0.04 \text{ mm}^2$) than intermittent delivery ($0.07 \pm 0.03 \text{ mm}^2$) and also formed woven bone ($0.42 \pm 0.23 \text{ mm}^2$) (Table 4). The lamellar bone should be maintained upon treatment withdrawal^{5,15} and depending on the environment, the woven bone could be partially remodeled into lamellar bone.

Continuous PGE₂ administration activated negative endosteal (trabecular and endocortical) bone balance – an imbalance of resorption and formation in favor of resorption; nevertheless, it stimulated bone formation rates above controls. Three mg PGE₂/kg/d stimulated trabecular and endocortical bone formation by 42% (Table 1) and 5.2-fold (Table 4), respectively, over controls. Compared to intermittent administration, continuous treatment was 30% less potent in elevating the trabecular bone formation rate (Table 5) and 1.8 times more potent in elevating the endocortical bone formation rate (Table 6).

If we could sustain continuous PGE₂ effects on the periosteal surface but eliminate its undesirable elevation of bone remodeling on the endosteal surface, then we might be able to achieve a positive bone balance. Shen et al. proposed the possibility that the continuous elevation of PTH could exert anabolic effects on skeletal tissues if its catabolic component can be minimized^{35,36}. They followed it with a study combining administration of continuous PTH with estrogen and found this combination to be a potent stimulator of bone formation increasing vertebral cancellous bone and cortical width³⁵⁻³⁷. However, studies using a combination of continuous PGE₂ with anti-catabolic agents have not yet been performed. Pre-treatment or co-treatment with anti-catabolic agents [risedronate and droloxifene (SERM)] did not blunt the anabolic effects of intermittent PGE₂^{28,29,38,39} nor were they superior to the PGE₂ alone^{28,40}. A recent study found that co-treatment with intermittent PTH and osteoprotegerin or alendronate increased its anabolic effect on the skeleton of oophorectomized mice⁴¹. These results suggest that possibly the same beneficial result may occur employing continuous PGE₂ administration.

Based on the above publications that anti-catabolic agents do not seem to blunt the anabolic actions of PTH or PGE₂, we propose that continuous PGE₂ administration may be able to achieve a positive bone balance if it is used with the anti-resorptive agents. Figure 3B shows continuous PGE₂ will result in endosteal (cancellous and endocortical) bone loss, stimulated periosteal and endosteal bone formation, negative endosteal bone balance and increased cortical porosity from intracortical remodeling. The intracortical porosity is localized near the endocortical surface and may have little influence on bone mechanics. Pores known to be close to endocortical surfaces have less effect on mechanical properties than pores near the periosteal surfaces⁴². Nevertheless, the combination of the above will result in reduced bone strength. We postulate that in combining continuous PGE₂ with an anti-catabolic agent, that the anti-

catabolic agent will depress bone resorption and not blunt or slightly blunt the stimulated bone formation to generate a positive bone balance. We propose that this combination treatment will affect cortical and cancellous bone as follows: (1) the stimulated periosteal and endosteal bone formation and increased intracortical porosity would persist; and (2) the negative cancellous and endocortical bone balances would be reversed to positive bone balances. The result is an increase in cancellous and cortical bone mass and strength (Figure 3B). On withdrawal or cessation of treatment, the combination treatment-induced bone gain will be maintained and the cortical bone mass will further increase as intracortical pores and cancellous remodeling spaces are filled in by bone formation^{5,43}. The net result is a skeletal tissue of superior bone strength compared to that resulting from intermittent PGE₂ administration due to the induction of a massive expansion of the periosteal surface by continuous PGE₂ (Figure 3C).

Since PGE₂ has some undesirable side effects, it is not a viable candidate to be employed as the above strategy in the clinic. However, continuous PTH may be substituted for PGE₂ if it is as powerful as PGE₂ in stimulating periosteal bone formation. In addition, further work is needed to determine if shorter infusion periods will be adequate to generate the beneficial periosteal expansion response. Regardless, further work with continuous PGE₂ and anti-catabolic models would improve our knowledge on mechanisms of expanding the periosteal perimeter to improve bone strength.

In summary, the findings in the current study demonstrate that PGE₂ stimulates both periosteal and endosteal bone formation by both continuous and intermittent administration. Whether net bone gain or loss happens is dependent upon the route of administration. Continuous PGE₂ treatment induces bone loss, while intermittent PGE₂ administration induces bone gain. Continuous infusion decreases cancellous bone mass and architecture by negative cancellous bone balance – with the increase in bone resorption exceeding the increase in bone formation. In cortical bone, continuous PGE₂ administration does not induce positive bone gain, as increased endocortical bone remodeling and intracortical porosity offset the stimulated periosteal expansion. In contrast, intermittent administration increases cancellous and cortical bone by stimulating both periosteal and marrow trabecular bone formation and has a positive endosteal bone balance where bone formation exceeds bone resorption. Continuous PGE₂ infusion is a more potent way to increase bone turnover or remodeling, bone resorption, periosteal and endocortical bone formation, but is less effective in increasing trabecular bone formation compared to intermittent treatment. We speculate that superior bone mass and strength might be achieved with co-treatment of continuous PGE₂ administration with an anti-catabolic agent. This supposition couples the highly beneficial periosteal expansion resulting from continuous PGE₂ treatment with suppression of endosteal bone remodeling with anti-catabolic agents. Potential for this combination could yield improved bone strength and reduced fracture risk and warrants further study.

In conclusion, both continuous and intermittent PGE₂ administration stimulated periosteal and endosteal bone formation, but the former lost and the latter gained bone and strength. Continuous PGE₂ treatment was a more powerful stimulator of periosteal apposition than was intermittent treatment. The combination of continuous PGE₂ with an anti-catabolic agent that converts negative endosteal bone balance to positive and maintains the stimulated periosteal apposition response may generate a larger increase in bone mass and strength.

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