

Increased Concentrations of Prostaglandin D₂ During Post-Fracture Bone Remodeling

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ABSTRACT. Objective. To test the hypothesis that increased concentrations of prostaglandin D₂ (PGD₂) correlate with bone remodeling. Studies using isolated bone cells indicate that PGD₂ may be implicated in the regulation of bone homeostasis, with a positive influence on bone anabolism. We studied patients with traumatic fractures and age- and sex-matched healthy controls as an *in vivo* model of increased bone remodeling.

Methods. Thirty-five patients with bone fracture and matched controls were recruited. Urine and sera samples were collected. Urinary 11 β -PGF_{2 α} , a PGD₂ metabolite, and PGE₂ metabolites (PGEM), serum lipocalin-type PGD₂ synthase (L-PGDS), bone alkaline phosphatase (bone ALP), and crosslinked C-telopeptides of type I collagen (CTX) were measured.

Results. At 5–6 weeks post-fracture, 11 β -PGF_{2 α} , L-PGDS, bone ALP, and CTX were significantly increased in the fracture patients compared to controls. PGEM levels were not different between groups. Levels of 11 β -PGF_{2 α} and bone ALP were positively correlated, suggesting that PGD₂ may be implicated in fracture repair.

Conclusion. These results support our working hypothesis that PGD₂ could be implicated in the control of bone anabolism in humans. (First Release Jan 15 2010; J Rheumatol 2010;37:644–9; doi:10.3899/jrheum.090622)

Key Indexing Terms:

BONE

PROSTAGLANDIN D₂

PROSTAGLANDIN SYNTHASE

FRACTURE REPAIR

BONE REMODELING

Fracture repair is a physiological process where inflammation, angiogenesis, and bone remodeling play important roles. A number of local and systemic factors influence the fracture repair process: these include cytokines, growth factors, and lipid mediators, which tightly regulate the action of cells responsible for bone healing^{1–3}.

Prostaglandins (PG) strongly influence the process of fracture repair, as demonstrated by delayed repair in mice null for cyclooxygenase-2 (COX-2)⁴ and by animal studies where COX inhibitors were used^{5,6}. However, despite the common use of nonsteroidal antiinflammatory drugs (NSAID) post-surgery and post-fracture, the effects of COX inhibition, if any, remain unclear during fracture repair in humans⁷. Some studies suggest that COX inhibition may delay fracture healing or increase the rate of non-unions^{8,9},

but prospective and controlled clinical trials are still lacking. PG may thus play a role in human bone metabolism, remodeling, and repair.

PG act on osteoblasts and osteoclasts, the cells responsible for bone formation and resorption, respectively¹⁰. PGD₂ is mostly known for its effects on the immune system and sleep^{11,12}, but recent evidence suggests new roles for this PG in bone physiology. We demonstrated that human osteoblasts produce PGD₂, and express the functional PGD₂ receptors D protanoid receptor type 1 (DP) and chemoattractant receptor-homologous molecule expressed on T helper cell type 2 (CRTH2). Activation of both receptors can lead to a different osteoblast phenotype, as DP receptor activation decreases osteoprotegerin (OPG) secretion, while CRTH2 increases osteoblast migration and decreases expression of RANKL (receptor activator of nuclear factor- κ B ligand)¹³. Moreover, PGD₂ and its metabolites are potent inducers of collagen synthesis by human osteoblasts in culture^{14,15}. Recently, we showed that PGD₂ inhibits both human osteoclast differentiation and mature osteoclast activity, reducing bone resorption *in vitro*¹⁶. These results suggest that PGD₂ may have a physiological role in bone remodeling, favoring bone anabolism.

PGD₂ is produced by 2 terminal PGD₂ synthases: hematopoietic-type synthase (H-PGDS) or lipocalin-type (L-PGDS) synthase. H-PGDS is strongly expressed by immune cells, while L-PGDS is mostly expressed in the

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brain and heart¹⁷. In addition to PGD₂ synthesis, L-PGDS also acts as a transporter for small lipophilic ligands such as retinoids and thyroid hormones. Altered expression of L-PGDS has been related to detached retinas¹⁸, atherosclerosis¹⁹, sleep disorders²⁰⁻²², and impaired renal function in diabetes^{23,24}. However, no evidence in the literature links this enzyme to human bone metabolism.

Our study is based on the hypothesis that PGD₂ is an important anabolic autacoid implicated in bone repair, as suggested by the *in vitro* studies using isolated osteoblasts and osteoclasts. Should this hypothesis be true, we would expect PGD₂ production to be increased *in vivo* during situations of increased bone remodeling. We studied patients with traumatic fractures and compared them to healthy age- and sex-matched controls as a model of increased bone turnover to test this hypothesis. Specifically, we determined the levels of a stable PGD₂ metabolite and of L-PGDS in patients within 5–6 weeks post-fracture, a period of active bone remodeling, and compared these levels to healthy controls.

MATERIALS AND METHODS

Patients. Patients with traumatic bone fracture were recruited 5–6 weeks post-fracture at the Centre Hospitalier Universitaire de Sherbrooke (CHUS) in collaboration with the Division of Orthopaedic Surgery during their routine visit at the hospital. Patients gave informed consent and the study was approved by the institution's Ethics Review Board. Age-, sex-, and weight-matched healthy controls were recruited at the same center and Bishop's University (Sherbrooke, QC, Canada). Patients and controls were excluded from the study if they used NSAID or corticosteroids less than 7 days before enrolment, if they had ever received calcitonin, bisphosphonates or chemotherapy treatments, or if they suffered from kidney failure, diabetes or asthma.

Blood and urine samples. Urine and blood samples were collected at the time of recruitment. Urine samples were immediately centrifuged at 1500 ×g for 15 min and the supernatants were frozen at –80°C until analyzed. Whole blood was drawn by standard venipuncture without anticoagulants, allowed to coagulate for 30 min at room temperature, and the serum was separated by centrifugation at 1500 ×g for 15 min. Serum aliquots were frozen at –80°C until analyzed.

Biochemical markers. PGD₂ production was assessed by measuring the levels of the PGD₂ primary stable metabolite 11β-PGF_{2α} in urine samples using enzyme immunoassay (EIA) kits (Cayman Chemical Co., Ann Arbor, MI, USA) with a detection limit of 5.5 pg/ml. This assay specifically recognizes 11β-PGF_{2α} with less than 0.01% cross-reaction towards PGD₂ or PGE₂.

PGE₂ production was assayed by measuring a stable PGE₂ metabolite (PGEM) after chemical derivatization of PGE₂ and its primary metabolites, namely 13,14-dihydro-15-keto PGE₂ and 13,14-dihydro-15-keto PGA₂, in the standards and urine samples to the single PGEM compound. PGEM concentrations were determined using commercial EIA kits (Cayman Chemical) following the manufacturer's protocol, with a detection limit of 2 pg/ml.

Lipocalin-type PGD₂ synthase expression was determined in the serum using the Prostaglandin D Synthase (lipocalin-type; human) EIA dosage kit (Cayman Chemical; detection limit 2 ng/ml), according to the manufacturer's protocol.

Bone formation occurring during fracture repair was assessed by serum bone-specific alkaline phosphatase (bone ALP) measured by commercial ELISA (Metra® BAP, from Quidel, distributed by Medicorp Inc., Montreal,

QC, Canada) on thawed serum aliquots. This ELISA detects as low as 0.7 U/l bone ALP with 3%–8% cross-reactivity with liver ALP and 0.4% towards intestine ALP.

To assess bone resorption, serum C-terminal telopeptides of type I collagen (CTX) were measured using the Serum CrossLaps® ELISA (Nordic Bioscience Diagnostics; Medicorp Inc., Montreal, QC, Canada). This ELISA has a detection limit of 0.020 ng/ml of CTX in a linear range up to 3.380 ng/ml.

Urine creatinine was measured (Vitros 950 System Chemistry, Ortho Clinical Diagnostics, Johnson & Johnson) and used to correct urine PG values.

Statistical analyses. Statistical analyses were performed using GraphPad Prism version 5.01. The Wilcoxon signed-rank test was used to compare patients to paired controls, while nonparametric Spearman correlations were used between markers. Data are shown as means ± standard error. Results were considered significant when *p* < 0.05.

RESULTS

Our study was designed to assess possible changes in the stable PGD₂ metabolite 11β-PGF_{2α} and L-PGDS in patients during the active bone formation phase of fracture repair. A cohort of 35 patients and matched controls was enrolled at our local hospital. No demographic differences were observed between the patients and controls (Table 1).

Prostaglandin D₂ pathway is specifically increased in fracture repair. To assess PGD₂ production, we measured the concentration of 11β-PGF_{2α} in urine samples. As shown in Figure 1, patients with fractures showed higher levels of 11β-PGF_{2α} in urine compared to controls (77.4 ± 8.8 vs 49.5 ± 3.9 ng/mmol creatinine, respectively; *p* = 0.0023). Figure 2 shows that serum L-PGDS in patients with fracture was significantly higher than that in controls (0.613 ± 0.03 vs 0.536 ± 0.03 μg/ml, respectively; *p* = 0.027). Since increases in PGD₂ and L-PGDS could have resulted from nonspecific inflammation associated with the fracture we also determined the concentration of stable PGE₂ metabolite, PGEM, in our population. As shown in Figure 3, PGEM did

Table 1. Demographic data of the study cohort.

Feature	Controls	Patients
No.	35	35
Sex, M/F	17/18	17/18
Age, yrs, mean (range)	34.8 ± 11.9 (20–56)	35 ± 11.8 (18–54)
Weight, kg, mean (range)	68.4 ± 11.6 (49.9–97.5)	68.3 ± 14.1 (44–118)
Fracture type		
Long bones		N = 25 (71%) Tibia/fibula 10 Radius/ulna 7 Humerus 4 Femur 2
Other		N = 10 (29%) Polytrauma 2 Hand/foot 4 Vertebrae 3 Hip 2 Scapula 1

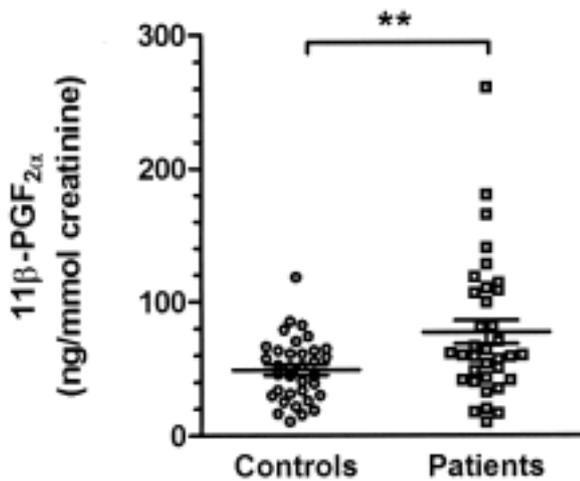


Figure 1. Urine 11 β -PGF_{2 α} was measured in patients and controls and corrected for creatinine. Data are means \pm SEM. **p < 0.01.

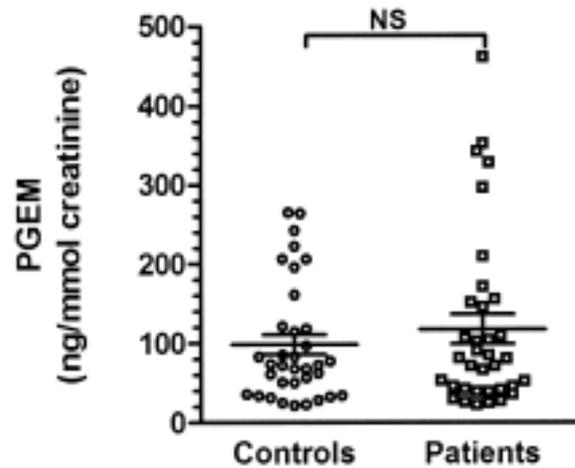


Figure 3. Urine PGE₂ metabolite (PGEM) was measured in patients and controls and corrected for urine creatinine. Data are means \pm SEM. NS: not significant.

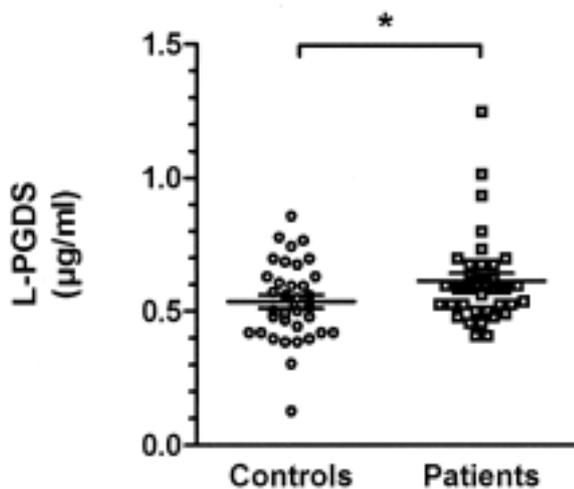


Figure 2. Serum lipocalin-type PGD₂ (L-PGDS) synthase was measured in patients and controls. Data are means \pm SEM. *p < 0.05.

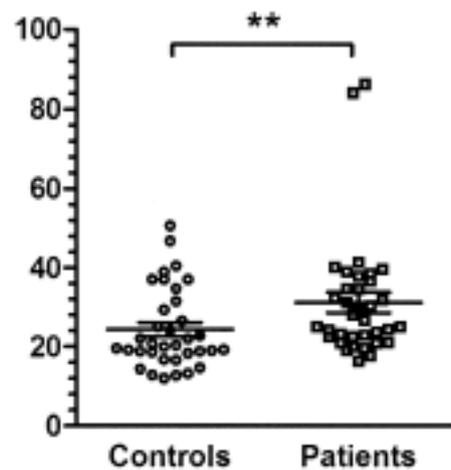


Figure 4. Serum bone ALP was measured in patients and controls. Data are means \pm SEM. **p < 0.01.

not differ significantly between control and patient groups (98.7 ± 12.5 vs 118.0 ± 18.8 ng/mmol creatinine, respectively; $p = 0.3633$), therefore arguing against a nonspecific overall increase in PG production.

Bone markers. Fracture repair is a process where both bone formation and resorption occur. Serum bone ALP and CTX were measured to assess these 2 phenomena, respectively. As expected, level of serum bone ALP was significantly higher in patients compared to controls (31.3 ± 2.6 U/l vs 24.4 ± 1.7 U/l, respectively; $p = 0.0033$; Figure 4). A strong increase in the bone resorption marker CTX was also observed in patients compared to controls (0.568 ± 0.07 ng/ml vs 0.301 ± 0.05 ng/ml, respectively; $p = 0.0002$; Figure 5), thus confirming active bone remodeling in the fracture group.

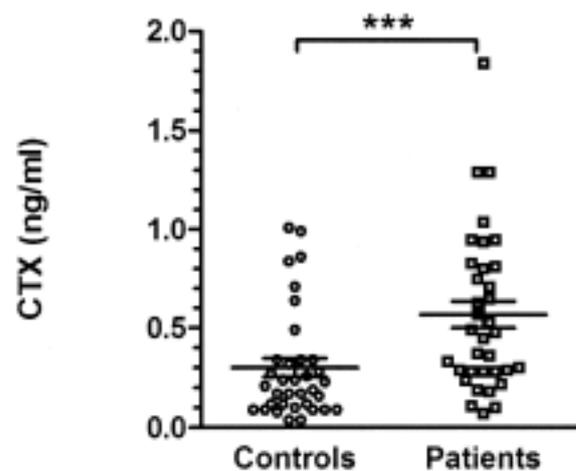


Figure 5. Serum CTX was measured in patients and controls. Data are means \pm SEM. ***p < 0.001.

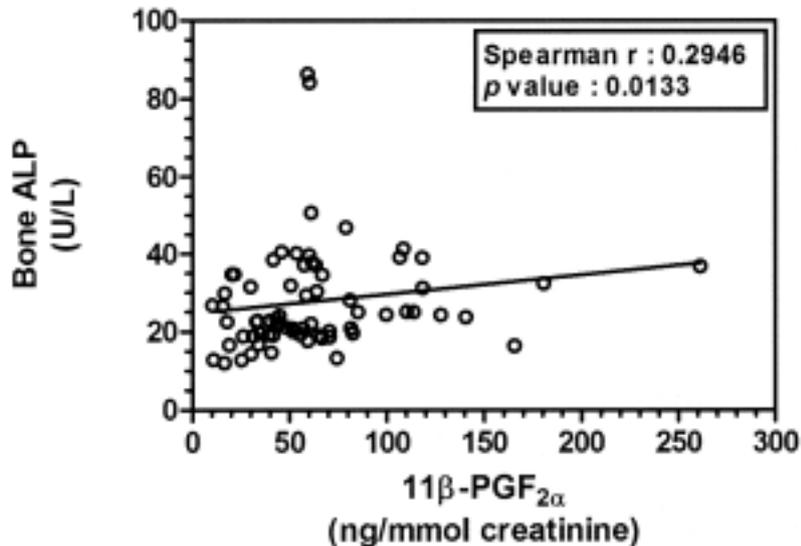


Figure 6. Spearman's rank test was performed to test correlation between 11β -PGF $_{2\alpha}$ and bone ALP in the whole cohort (N = 70).

Correlation of PGD $_2$ metabolite and bone formation marker. To determine if the higher production of the PGD $_2$ metabolite 11β -PGF $_{2\alpha}$ in the patient group would correlate to an increase in the bone remodeling process, we tested whether serum bone ALP correlated with urine 11β -PGF $_{2\alpha}$. As shown in Figure 6, bone ALP and 11β -PGF $_{2\alpha}$ levels were positively correlated (Spearman $r = 0.2946$, $p = 0.0133$). In contrast, no correlation was observed between 11β -PGF $_{2\alpha}$ and CTX. The expression of L-PGDS and level of PGEM were not correlated with any marker measured.

DISCUSSION

Bone metabolism is a complex and tightly controlled process implicating several cytokines, growth factors, and soluble mediators such as prostaglandins^{1,3}. Inadequate control of the balance between bone production and resorption, either locally or systemically, is an important pathophysiological sequence in different diseases such as rheumatoid arthritis²⁵, spondyloarthritis²⁶, osteoarthritis²⁷, periodontitis²⁸, and osteoporosis²⁹, to cite a few. Better understanding of the different elements implicated in this control may lead to identification of new pharmacological targets for treatment of these diseases.

Evidence in the literature from our group^{13,16} and others^{14,15} suggests that PGD $_2$ may have a positive influence on bone metabolism, increasing bone formation and inhibiting bone resorption. Our objective was to test *in vivo* the hypothesis that increased levels of PGD $_2$ would be associated with increased bone remodeling using traumatic bone fracture in humans as an experimental model.

Fracture repair is a physiological event regulated by local and systemic factors. Immediately after a fracture, the microenvironment of the damaged site is drastically

changed. Formation of hematoma and inflammation are the first events to occur. During this period, leukocytes are attracted to the wound site, where they secrete various mediators modulating the inflammatory response, including cytokines and PG. While this inflammatory response lasts only a few days, PG-mediated effects may last longer as they remain actively produced by bone and surrounding cells^{13,30,31}.

The importance of PG in the process of fracture repair is well characterized in animal models. COX-2 was shown to be essential to fracture repair^{4,6}, while PGE $_2$ receptors EP2 and EP4 were shown to be implicated in murine fracture repair^{32,33}, but the role of PGD $_2$ in fracture repair and bone remodeling remains unknown.

We investigated if L-PGDS expression and the stable urinary PGD $_2$ metabolite 11β -PGF $_{2\alpha}$ were changed during the process of bone remodeling and if they correlated with markers of bone formation and resorption. 11β -PGF $_{2\alpha}$ is the major PGD $_2$ metabolite produced *in vivo* and is considered a physiological marker of PGD $_2$ production: it has been used to assess mast cell activation and PGD $_2$ production during allergic and asthmatic responses³⁴⁻³⁶. We demonstrated that 11β -PGF $_{2\alpha}$ is significantly increased during fracture repair. To our knowledge, this is the first time that PGD $_2$ has been linked to bone metabolism in humans.

To test whether this key enzyme was implicated in bone remodeling, serum L-PGDS was measured in patients with fractures. Like 11β -PGF $_{2\alpha}$, L-PGDS was significantly increased during fracture repair. It is interesting that slight changes in serum L-PGDS can be a predictor or marker of pathologies such as coronary heart disease¹⁹, hypertension³⁷, sleep apnea²⁰, and diabetes^{23,24}. We cannot rule out

the possible contribution of the other PGD₂ synthase, H-PGDS, in the process, but technical limitations prevent measurement of this intracellular enzyme in blood or other body fluids.

PGD₂ is a key component of the inflammatory response^{38,39}. Therefore, all patients were studied during an orthopedic followup visit 5 to 6 weeks post-fracture, a time at which inflammation is not considered to be a major component of the repair process⁴⁰ and when bone remodeling starts to occur⁴¹. We found no significant difference in the stable PGE₂ metabolite in patients compared to controls. This suggests that the increased 11β-PGF_{2α} levels observed in patients were not due to a general increase in PG synthesis associated with inflammation. Since osteoblasts were shown to produce PGD₂, it is tempting to hypothesize that osteoblasts are responsible for the increased synthesis of PGD₂ found in patients with fracture; this study, however, was not designed to allow identification of the cellular origin of PGD₂.

Bone remodeling is often measured by biomarkers of bone turnover. During fracture repair, both endochondral and intramembranous bone formation occur, leading to formation of a bony and cartilaginous callus, which will later be resorbed in order for the bone to regain its previous integrity, resistance, and shape. Activation of osteoblasts and osteoclasts is critical for the completion of fracture repair. We measured serum levels of bone ALP, as a marker of bone formation and osteoblast activation, as well as serum CTX, to assess bone resorption. Concentrations of both markers were increased in the fracture patients compared to controls, indicating that there was significantly increased bone turnover in these patients. This confirms the premise that increased bone remodeling is occurring in the studied population. To test our hypothesis that PGD₂ is increased during bone remodeling, we tested if 11β-PGF_{2α} correlated with levels of either bone ALP or CTX. Our analysis showed a significant and positive correlation with bone ALP and no correlation with CTX. Moreover, no correlation was found for the bone markers and PGEM, thus underlying the specificity of PGD₂ production during fracture repair.

Our results show the first evidence that a specific prostaglandin pathway, PGD₂ and its lipocalin-type synthase, is activated during fracture repair in humans. Moreover, we demonstrated that PGD₂ production correlates with a classic osteoblast activation and bone formation marker. These findings support our hypothesis that PGD₂ may be an important player in bone physiology and that it may constitute an important and biologically relevant anabolic stimulus for bone.

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