In Situ Microdialysis in Bone Tissue

Stimulation of Prostaglandin E_2 Release by Weight-bearing Mechanical Loading

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Abstract

In this study we describe, to our knowledge, the first experiment using the microdialysis technique for studying the release of prostaglandin E_2 (PGE_2) in the proximal tibia metaphysis secondary to mechanical loading. Nine healthy females, six in the loading group and three controls, mean age 34±2 (years±SEM), participated. A standard microdialysis catheter was inserted into the tibia metaphyseal bone under local anesthesia. Samplings were done every 15 min under a 2-h equilibration period. Thereafter, heel-drops (one impact per second) with as hard impact of the heels into the floor as possible, were done for 5 min by the subjects in the loading group. The control group performed no exercise. Sampling continued after this for another 2-h period.

Basal levels of PGE_2 in the proximal tibial metaphysis were determined to a mean of 45±10 pg/ml (mean±SEM, $n = 6$). The major finding was a 2.5–3.5-fold increase in the release of PGE_2 after mechanical loading. The increase was statistically significant ($P < 0.05$ compared with basal levels) 1 h after the mechanical loading and persisted for the rest of the experimental period. No major alterations were observed in the control group. In conclusion, our data demonstrate that in situ microdialysis is a useful method for studying the PGE_2 production in human bone. Furthermore, a rapid and significant increase in PGE_2 levels was noticed in response to dynamic mechanical loading. (*J. Clin. Invest.* 1996. 98:2446–2449.) Key words: physical exercise • mechanical loading • bone metabolism • microdialysis • prostaglandins

Introduction

Osteoporosis with associated fragility fractures is a disorder affecting ~ 40% of the women and 15% of the men during the rest of their lifetime after age 50 in the United States (1). Decrease in habitual physical activity is probably one of the main reasons for the decrease in bone mass and associated increase in rates of hip fractures observed during the last 30 yr (2, 3).

Although it is well known that bone can adapt to the load applied to it, the mechanism by which bone detects and converts mechanical load into a biological response is a matter of speculation (4). Osteocytes are considered important mechanosensors of the bone (5). PGE_2 is believed to play a important role in the recruitment of mesenchymal (osteoblastic) precursor cells (6) as well as osteoclastic cells in bone marrow (7).

In simulating mechanical stress in vitro, fluid flow has been shown to stimulate PGE_2 production in osteocytes, and to a minor degree also in osteoblasts (8). The avian ulna model has shown in vivo that a brief single episode of mechanical loading is sufficient to induce bone formation (9, 10), while the osteogenic response to a single strain change is reduced after pretreatment with indomethacin (11). That enhanced prostanoid biosynthesis may be involved in anabolic events in bone in response to mechanical loading is supported by experimental studies in vitro where dynamic mechanical loading in perfused cores of canine bone is accompanied by release of PGE_2 and PGI_2 (prostacyclin) within 5 min after the beginning of loading (12).

The microdialysis technique was initially developed for studying the metabolism of subcutaneous tissue as well as for studying neurobiology, primarily in animals (13–17). However, the technique can be adapted to almost any tissue of the body and allows continuous measurements of concentrations in vivo of virtually any substance of interest with molecular size below the cutoff limit of the dialysis membrane. Prostaglandins (size 0.3–0.5 kD) are well suited to be examined by the microdialysis technique. Here, we describe, to our knowledge, the first experiment using the microdialysis technique for studying the bone metabolism in humans, by determining the production of PGE_2 in the proximal tibial metaphysis in younger females and the PGE_2 response to mechanical loading.

Methods

Nine healthy females, six in the mechanical loading group and three in the control group, mean age 34±2 (years±SEM), with regular menses and on no medication, participated. They were recruited after advertising at the local hospital. The study protocol was approved by the Ethical Committee of the Medical Faculty, University of Umeå

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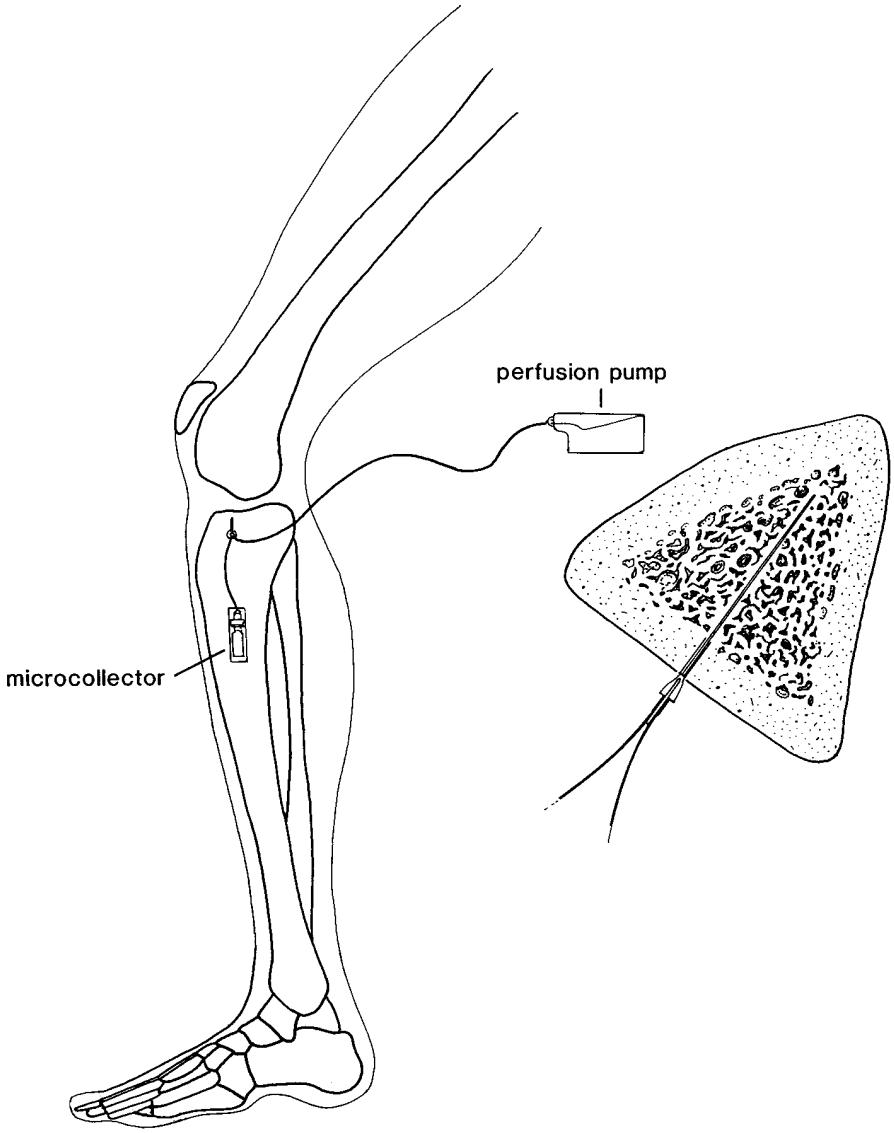

Figure 1. The lower extremity with the connected microdialysis system (*left*) and a cross-section of the proximal tibia with the microdialysis catheter introduced (*right*).

and the experiment was conducted according to the principles expressed in the Declaration of Helsinki.

After disinfection of the skin over the antero-medial aspect of the tibia proximal to the insertion of the pes anserinus, local anesthesia with 8–10 ml prilocainhydrocloride + adrenalin (Citanest® adrenalin 10 mg/ml + 5 μg/ml; Astra, Södertälje, Sweden) was induced in the skin, subcutaneous tissue, and the periosteum. A small skin incision was done and a 2-mm hole (depth ~ 50 mm) was drilled in the tibia. A metallic cannula, length 1.0 cm, outer diameter 2 mm, was then introduced in the cortical part of the metaphysis to protect the catheter from cortical swelling and to minimize bleeding. Thereafter, the microdialysis catheter, with a diameter of 1.4 mm and a length of the membranous covered active part of 30 mm, was introduced into the cancellous bone and fixed to the skin (Fig. 1).

The microdialysis system consists of a battery-driven infusion pump (CMA 106; CMA/Microdialysis AB, Stockholm, Sweden) with a fixed infusion rate at 0.3 μl/min. The dialysis catheter (CMA 60; CMA/Microdialysis AB), perfused with Ringer acetate, has a pore dimension of 20 kD and at the given perfusion rate almost full recovery of PGE_2 is achieved in the dialysate (17). The void volume from the probe to the sample collector is 3 μl, equivalent to a 10-min fraction (Fig. 1). After flushing the system, sampling was done every 15 min and every dialysis sample (4.5 μl) was diluted to 25 μl and immediately frozen to −70°C. After a 2-h equilibration period, the subjects in the mechanical loading group, wearing jogging shoes, performed heel-drops with as hard impact of the heels onto the floor as possible for 5 min, one impact per second. Thereafter, sampling continued for another 2-h period. Samples from two consecutive 15-min periods were pooled and PGE_2 analyzed using a commercially available PGE_2 radioimmunoassay kit (DuPont, Boston, MA). Samples or standards, together with ^{125}I-PGE_2 as tracer, were incubated with rabbit anti-PGE_2 antibodies in a 0.0255 M phosphate buffer, pH 6.8, overnight at 4°C. The samples were then precipitated by adding polyethylene glycol, centrifuged, and decanted, and the radioactivity in the pellet was determined by using a gamma counter. The sensitivity of the assay is 10 pg/ml. The antibody used shows a 3.7% cross-reactivity to PGE_1 but $< 1\%$ to dehydro-keto-PGE_2, PGA_2, 6-keto-$PGF_{1\alpha}$, PGF_2, and TXB_2.

To quantify the load applied to the heel and foot during heel-impacts, pressure measurements using the F-scan foot pressure measurement system (18) were done, exactly simulating the loading conditions in the experiment. These measurements were performed at a mean of 6 wk after the microdialysis experiment. Since the subjects did not experience any pain or discomfort of the inserted catheter

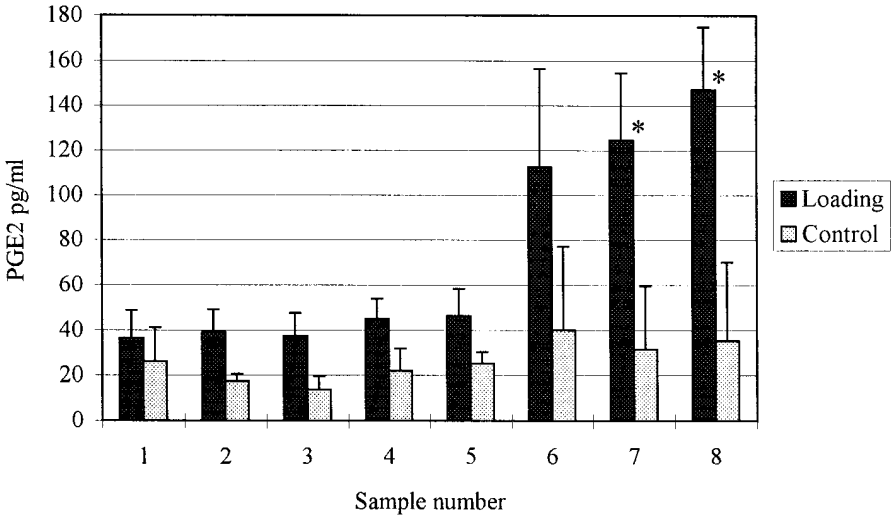

Figure 2. PGE_2 in dialysate. In the loading group samples 1–4 are before loading while samples 5–8 are after loading, $*P < 0.05$ compared with the basal level in the group. Mean±SEM shown. Each sampling period is 30 min, $n = 6$ in the loading group and $n = 3$ in the control group.

during loading, no influence of an applied catheter on the impact of mechanical loading was to be expected. Therefore, the pressure registrations were done without a catheter inserted.

For the statistical analysis, the SPSS software (SPSS Inc., Chicago, IL) was used. The analysis was Student's paired *t* test comparing the mean basal level (sample 4) and the various samples after the 2-h equilibration period. No multiple comparisons were done. $P < 0.05$ was considered significant.

Results

The results of the experiments are shown in Fig. 2. In the experimental group ($n = 6$), the basal level of PGE_2 in the interstitium of cancellous bone in the proximal tibia during the initial unloaded period was determined to be 45±10 pg/ml (mean±SEM) in the sample closest to mechanical loading (sample 4). The PGE_2 concentration in these unloaded bones showed very small variation at the different preloading sample times. Subsequent to mechanical loading, a 2.5–3.5-fold increase in PGE_2 release was noticed. The increase was statistically significant ($P < 0.05$ compared with basal levels in the loading group) 1 h after the mechanical loading and persisted for the rest of the experimental period.

In the control group ($n = 3$), the mean level of PGE_2 during the eight sampling periods was 27±17 pg/ml (mean±SEM) and no major changes were registered during the experimental period of 4 h (Fig. 2).

When analyzing the pressure measurements of the F-scan, the peak pressure on the heel of every heel-drop was determined to 226±25 kPa (mean±SEM).

Discussion

In this paper, we describe a microdialysis technique for studying PGE_2 production in human bone in situ and a rapid increase in the PGE_2 production rate secondary to weight-bearing mechanical loading in younger females.

In using the microdialysis technique for investigating dynamic courses in human bone, several difficulties must be considered and overcome. To minimize the risk for infection, an operating room with facilities is necessary to ensure strict sterility when introducing the microdialysis catheter. The catheter is very soft and the handling and insertion must be done carefully to avoid damage of the soft membrane surrounding the catheter due to contact with bone trabeculae. The risk for clotting of the catheter has to be considered in relation to insertion time and the small perfusion volume. Therefore, the flushing of the system has to be done without delay after insertion of the catheter in the bone. Finally, the samples must be frozen to −70°C immediately because of the rapid metabolism of PGE_2. With the given perfusion rate, this makes the samples size quite small, but in this experiment PGE_2 could be measured in all samples. Using a low perfusion rate, almost full recovery of PGE_2 can be expected in the dialysate. This indicates that the measured concentrations of PGE_2 in the dialysate are most likely close to the concentration of PGE_2 in the proximal tibial metaphysis.

We chose the proximal tibial metaphysis for insertion of the microdialysis catheter for several reasons. Entrance to the bone is easy on the medial aspect of the proximal tibia in humans, and the relation in size between the cortical and the cancellous part of bone is optimal for applying the standard microdialysis catheter so that the membrane part (30 mm) is totally embedded in the proximal tibial metaphysis.

Applying a local anesthetic with adrenalin induces a local vasoconstriction. The influence of this on PGE_2 production cannot be elucidated from this experiment. The duration of local analgesia limits the time span of microdialysis in human bone in vivo. None of the investigated females had any significant pain after the experiment. However, after a period of 6–8 h after the application of the local anesthetics most of the individuals experienced a slight discomfort when loading, which persisted for a couple of days after the experiment. All of the skin incisions healed without complications and no infections occurred.

Theoretically, the tissue trauma when preparing the bone for insertion of the catheter may affect the production rate of PGE_2 and, therefore, the measured concentrations may not represent the normal physiological levels. However, since the levels of PGE_2 were constant in the control group during the entire experimental period and during 2 h before loading in the other group, it seems reasonable to conclude that the tissue disturbances caused by the insertion of the probe were of minor importance. There were rather large interindividual levels

of PGE_2 production which might explain the generally lower levels of PGE_2 observed in the small control group.

Prostaglandins are considered potent regulators of bone formation as well as bone resorption (19–22). They are very likely to play a role in the cascade events transforming the mechanical signal to a cellular response (8, 23). However, although we demonstrate a significant enhancement of PGE_2 concentration in proximal tibia subsequent to mechanical loading, the net result on bone metabolism cannot be elucidated from this experiment. It has been shown that osteoblastic bone formation caused by low frequency mechanical loading can be prevented by inhibition of prostaglandin biosynthesis (11). Therefore, it is tempting to speculate that the rise of PGE_2 in our subjects may be causally involved in anabolic events due to mechanical loading.

Microdialysis in situ does not identify the cellular source of PGE_2 in the human bone. Several cell types, separately or in concordance, are possible contributors to the production of PGE_2 in bone tissue. Osteocytes, osteoblasts, preosteoblasts, marrow cells, endothelial cells, or other interstitial cells are candidates for production of prostanoids in the bone. Furthermore, the precise action of PGE_2 and the cells responding to PGE_2 have not yet been entirely identified.

Most interestingly, in relation to our findings, pulsating fluid flow stimulates PGE_2 production and induces an increase in PGHS-2 (prostaglandin G/H synthase 2) mRNA expression in a culture of primary mouse bone cells (24). Similar to our findings, the response to mechanical stress persisted after removal of the stress (24). In our experiment, the concentration of PGE_2 in the proximal tibial metaphysis was still increasing compared with basal levels 2 h after the mechanical loading. We suggest that the PGE_2 release initiated by the mechanical loading is enhanced and maintained even after the end of mechanical loading in human tibial metaphyseal bone. Further experiments are needed to elucidate if induction of PGHS-2 is involved in the amplification of the initial response in human bone tissue secondary to mechanical loading.

In conclusion, in situ microdialysis seems to be applicable for studying the synthesis of PGE_2 in human bone. Basal levels of PGE_2 in human bone were established and a rapid and significant increase in PGE_2 levels was noticed after dynamic mechanical loading. In this first experiment, the few individuals of the control group did not perform any physical exercise at all. In ongoing experiments, the effect of weight-bearing loading on PGE_2 and PGI production is compared with the response to non–weight-bearing muscle exercise, or complete rest, in larger groups of individuals.

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