CHAPTER 4

INITIAL STRESS-KICK IS REQUIRED FOR FLUID SHEAR STRESS-INDUCED RATE DEPENDENT ACTIVATION OF BONE CELLS

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ABSTRACT

The shear stress induced by the loading-mediated flow of interstitial fluid through the lacuno-canalicular network is a likely stimulus for bone cell adaptive responses. Furthermore, the magnitude of the cellular response is related to the rate of mechanical loading rather than its magnitude. Thus, bone cells might be very sensitive to sudden stress-kicks, as occuring e.g., during impact loading. There is evidence that cells change stiffness under stress, which might make them more sensitive to subsequent loading. We studied the influence of a stress-kick on the mechanosensitivity of MC3T3-E1 osteoblast-like cells under different peak shear rate conditions, as measured by nitric oxide production. MC3T3-E1 bone cells were treated with steady or pulsating fluid shear stress (PFSS) for 5 min with different peak rates (9.70 Pa-Hz, 17.5 Pa-Hz, and 22.0 Pa-Hz) using varying frequencies (5 Hz, 9 Hz), and amplitudes (0.70 Pa, 0.31 Pa). PFSS treatment was done with or without fluid flow pre-treatment phase, which removed the initial stress-kick by first applying a slow fluid flow increase. Nitric oxide production in response to fluid shear stress was rate dependent, but necessitated an initial stress-kick to occur. This suggests that high-rate stimuli condition bone cells to be more sensitive for high-frequency, low-amplitude loads.

INTRODUCTION

During life, bones adapt their mass and structure to the prevailing mechanical loads, in order to resist mechanical failure with minimum material expense. It is currently believed that this process of adaptation is governed by osteocytes, which respond to the loading-induced flow of interstitial fluid through the lacuno-canalicular network (1). When bones are loaded, the resulting deformation will drive the thin layer of interstitial fluid surrounding the network of osteocytes within the calcified bone matrix to flow from regions under high pressure to regions under low pressure (2, 3). The loading-induced movement of labeled molecules directly demonstrated fluid flow in the mineralized matrix of bone both *in vivo* (4) and *ex vivo* (5). Osteocytes have been proven to be sensitive to this type of fluid shear stress (6). Subsequently, the osteocytes may signal the osteoblasts and osteoclasts to change their bone remodeling activities (1, 7).

Bone cells (osteocytes, and to a lesser extent also osteoblasts) respond to fluid flow stimulation *in vitro* (6, 8-15). They are sensitive to fluid shear stress (9, 16), rather than to streaming potentials mediated by the transport of ions with the flow (17, 18). Bone cells are also more responsive to shear stress by fluid flow stimulation than to direct mechanical strain by substrate stretching (19-21). Bone cells respond to fluid flow with increased nitric oxide (NO) and prostaglandin E_2 production, which are essential for the induction of new bone formation in reaction to mechanical loading *in vivo* (22, 23). In particular, we have recently found that MC3T3-E1 osteoblastic cells produce NO in response to fluid shear stress in a rate dependent manner (8). Since osteoblasts respond to fluid shear stress as osteocytes, although to a lesser extent, osteoblasts could provide a practical model for osteocyte response to fluid shear stress. However, how bone cell sensitivity is modulated by the various flow parameters (amplitude, frequency, duration, etc.) is not well understood.

The fluid shear stress amplitudes and frequencies in bone can be determined theoretically from known physiological loading parameters. By applying Biot's theory of poroelasticity to bone, the predicted range of *in vivo* fluid shear stress ranges from 0.8 – 3 Pa due to loading-induced strains ranging between 1000 – 3000 με (3, 24). Several *in vitro* studies (6, 8-15) have confirmed that this range of fluid shear stress magnitudes is able to stimulate bone cells.

It has been suggested that the rate (determined by the frequency and amplitude) rather than the magnitude alone of the applied loading stimulus correlates to bone formation (8, 25, 26). This implies that bone formation is enhanced by dynamic loading. Thus, both the magnitude (or amplitude) and the frequency of loading seem to be important parameters for bone formation. Indeed, it has been shown that low magnitude (< 10 με), high-frequency (10 – 100 Hz) loading is capable of stimulating bone growth and inhibiting disuse osteoporosis (27). Furthermore, it has been shown that high-amplitude, low-frequency stimuli are rare in the activities of daily life, whereas high-frequency, low-amplitude stimuli are common (28). The rate of loading seems to be a decisive factor in bone formation and maintenance. However, how bone cells respond to the rate of loading is not well understood.

High impact physical activity, including jumps in unusual directions, has a great osteogenic potential in humans (29) and in osteopenic ovariectomized rats (30). High impact drop jumps were shown to significantly increase bone formation rates compared to that of baseline walking (31). Furthermore, an initial high stress rate, as in step-wise increased fluid shear stress, was shown to stimulate neonatal rat calvarial bone cells (32). Therefore, the osteogenic response to high impact activity might be related to the response of bone cells to a sudden increase (i.e., stress-kick) in fluid shear stress. The osteogenic benefits of high impact activity might imply that the bone cell response to fluid shear stress is non-linear.

Tanaka and colleagues (33) showed that stochastic resonance enhances bone formation. Stochastic resonance is a phenomenon by which non-linear systems

are able to amplify a response to small periodic stimuli with the aid of noise (34). Stochastic resonance has been used to explain various phenomena in biological systems (34, 35) including the activity of cells under microgravity (36). During exercise, as in active sports, bone is subjected to quick variations in loading, which is equivalent to a noisy loading environment. Therefore, the possibility arises that bone cells are more responsive to fluid shear stress mediated by high impact activity compared to low impact activity. If so, the bone cell response to fluid shear stress might be non-linear, that is, requiring an initial stress-kick.

In this study, we investigated whether an initial stress-kick is required for the response of bone cells to varying rates of fluid shear stress. The initial stress-kick occurs during the quick transition from 0 to a non-zero stress initiating a fluid shear stress stimulation of cells. We used NO production as a parameter for bone cell activation since it is an early mediator of mechanical loading-induced bone formation (37), and it has been shown to be essential to adaptive bone formation *in vivo* (23).

MATERIALS AND METHODS

Bone cell cultures

MC3T3-E1 cells (Kodama, et al, 1981; kindly provided by Dr. Kumegawa, Mekai University School of Dentistry, Sakado, Saitama, Japan) were cultured up to near-confluency in 75 cm^2 cell culture flasks (Nunc, Roskilde, Denmark), using α-Modified Eagle's Medium (α-MEM; Gibco, Paisley, UK) supplemented with 10% fetal bovine serum (FBS; Gibco), ascorbate (50 μg/ml; Merck, Darmstadt, Germany), β-glycerophosphate disodium salt hydrate (10 mM; Sigma, St. Louis, MO, USA), l-glutamine (300 μg/ml; Merck), gentamycine (50 μg/ml; Gibco), fungizone (1.25 μg/ml; Gibco), at 37°C with 5% CO_2 in air. Cells were then harvested using 0.25% trypsin (Difco

Laboratories, Detroit, MI, USA) and 0.1% EDTA (Sigma) in PBS, and seeded at $2x10^5$ cells per polylysine-coated (50 μg/ml; poly-L-lysine hydrobromide; Sigma) glass slide (5 cm^2), then incubated overnight in α-MEM with 10% FBS to promote cell attachment prior to fluid shear stress experimental treatment as described below.

Parallel-plate flow chamber (PPFC) design for dynamic fluid shear stress

The PPFC was designed such that it is capable of inducing high-frequency fluid shear stress on the cells. This chamber was designed for dynamic flow regimes utilizing flow frequencies up to 11.2 Hz (the critical frequency, as discussed below). A dimensionless parameter αh ($\alpha = \sqrt{(\pi f \rho/\mu)}$, where f = flow frequency, ρ = fluid density, μ = fluid viscosity; h = distance between the plates, 300 μm; a more detailed derivation of this parameter has been done elsewhere, see (38, 39) determines the magnitude of the dynamic wall shear stress, with its oscillating component scaled by a dimensionless "shear factor" $T(\alpha h)$:

$$\tau_w(t) = \frac{6\mu}{bh^2} QT(\alpha h)\sin(\omega t + \psi)$$

where Q is the flow amplitude, $\omega = 2\pi f$, and ψ is the phase difference between the flow and the wall shear stress τ_w. When $T(\alpha h) = 1.0$, ψ vanishes and the flow and wall shear stress are always in phase giving a quasi-parabolic velocity profile throughout one flow period. A critical frequency f_c exists (when $\alpha h = 2$, f in α is the critical frequency), above which, the parabolic velocity profile does not occur (i.e., when $\alpha h > 2$). The quasi-steady flow regime for exposing cells to predictable levels of dynamic wall shear stress requires $\alpha h < 2$ based on the velocity profile and the magnitude of $T(\alpha h)$ (Fig. 1).

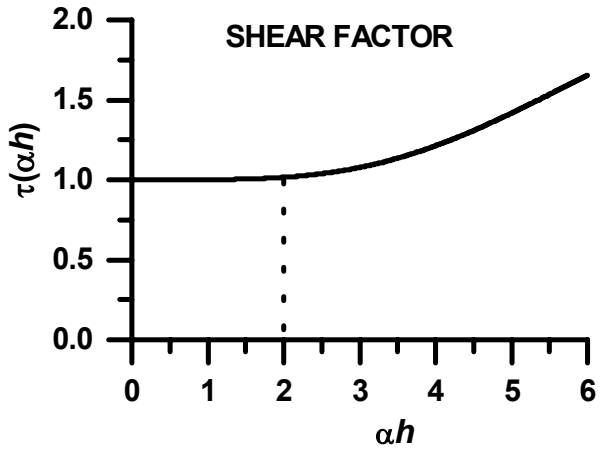

Figure 1. The shear factor predicts homogenous wall shear stress in the parallel-plate flow chamber. The shear factor $T(\alpha h)$ is equal to 1.0 when $\alpha h < 2$. Under this condition, the fluid flow regime is quasi-steady, which induces a homogenous wall shear stress.

Fluid shear stress

Pulsating fluid shear stress was generated using a flow apparatus containing a parallel- plate flow chamber (PPFC) as described earlier (6, 8). Briefly, fluid shear stress was induced on the monolayer of cells attached to the polylysine-coated glass slide serving as the bottom of the parallel-plate flow chamber, by circulating 9 ml of α-MEM containing 2% FBS plus supplements as described above using a computer-controlled micro-annular gear pump (developed by HNP Mikrosysteme GmbH, Parchim, Germany). Precise flow regimes were implemented by controlling the pump using computer-mediated instrumentation by LabView™ (National Instruments Corp., Austin, Texas, USA). The flow through the PPFC was monitored online using a small animal blood flowmeter (T206, Transonic Systems Inc., Ithaca, NY, USA).

During the shear stress experiment the flow apparatus was placed in a 37°C incubator, and connected to a gassing system that maintained a pH of 7.4 in the medium using 5% CO_2 in air. For the fluid shear stress experiments, the culture medium was changed to α-MEM with 2% FBS plus supplements as described above (fluid viscosity = 0.69 x 10^{-4} Pa-s). Cells were incubated for 5 min in the

presence of: 1) steady fluid shear stress (0.70 Pa; 0 rate), 2) low-amplitude pulsating fluid shear stress (PFSS, 0.70±0.31 Pa) at two different frequencies (5 Hz, with PFSS rate 9.70 Pa-Hz or 9 Hz, with PFSS rate 17.5 Pa-Hz), and 3) high-amplitude pulsating fluid shear stress (0.70±0.70 Pa) at 5 Hz, with PFSS rate 22.0 Pa-Hz, 4) no shear stress (static controls). Pa-Hz was the unit introduced for the fluid shear stress rate, which has a maximum value of $2 \pi \times$ fluid shear stress amplitude $\times$ frequency. Medium samples were taken after 5 min for measuring the response to fluid flow parameterized as production of NO in the medium.

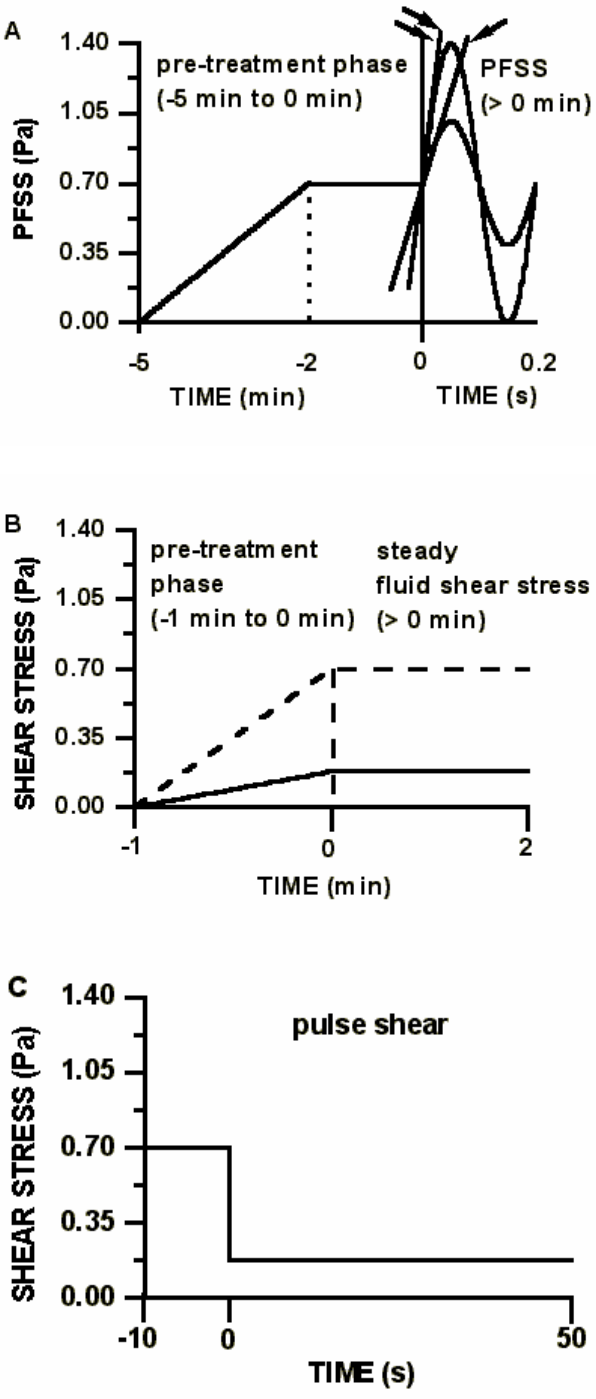

Figure 2. Fluid shear stress regimes applied to MC3T3-E1 bone cells for 5 min. A. The stress-kick was removed in the fluid shear stress regime by inducing a slowly rising shear stress from 0 to 0.70 Pa within 3 min, then a steady shear stress at 0.70 Pa for 2 min (5 min pre-treatment phase), followed by a pulsating fluid shear stress with an average of 0.70 Pa for 5 min (only 0.2 sec is shown). The slope of the lines shown equaled the peak fluid shear stress rate of the flow regime. The rate was increased by raising the flow regime amplitude from 0.31 Pa to 0.70 Pa (double arrows, 5 Hz high-amplitude; single arrow, 5 Hz low-amplitude). The peak fluid shear stress rate was also varied by changing the frequency. The steady flow regime was constant at 0.7 Pa, hence having zero rate. B. The initial stress kick at an earlier time point was removed by introducing a slow rise from 0 to 0.18 Pa (solid line) or 0.7 Pa (dashed line) (the 1 min pre-treatment phase) before applying a subsequent steady shear stress (0.18 or 0.7 Pa, respectively). C. A single pulse shear stress (i.e. a short duration stress-kick) was applied with an amplitude of 0.7 Pa for 10 sec followed by a constant 0.18 Pa for 5 min (only 50s is shown).

Removal of the initial stress-kick and application of a single pulse stress

The PFSS regimes applied give an initial stress-kick by the immediate rise of fluid flow to induce a 0.7 Pa fluid shear stress average. To remove the initial stress-kick, a pre-treatment phase for 5 min was introduced to the regimes. The same set of experiments was performed with a pre-treatment phase (Fig. 2A), composed of a linearly increasing flow from 0 to 8.3 ml/min to induce fluid shear stress from 0 to 0.7 Pa, within 3 min followed by a 2-min steady flow of 8.3 ml/min inducing a steady fluid shear stress at 0.7 Pa. Immediately after the 5 min pre-treatment phase, the cell monolayers were independently subjected to the same flow regimes described above. The shear rates ($2\pi \times$ amplitude $\times$ frequency) we used in our experiments were chosen due to their physiological relevance. We used 5 and 9 Hz as in a previous paper (8), where we calculated the frequency content of forces acting on a hip, and found that for jogging pace, the frequencies reach up to 9 Hz. Note that with the 5 min pre-treatment phase, the flow regime does not induce an initial stress kick as in the PFSS regimes.

To investigate the effect of the initial-stress kick at an earlier time point, cells were incubated with a 1 min pre-treatment phase before inducing a steady fluid shear stress. In these experiments, the initial stress-kick was removed by increasing the flow to induce a fluid shear stress from 0 to 0.18 Pa or from 0 to 0.7 Pa within 1 min. Immediately after the 1 min pre-treatment phase, a steady fluid shear stress followed to induce 0.18 Pa or 0.7 Pa, for 5 min (Fig 2B).

To investigate the effect of a single pulse shear stress, cells were incubated in the presence of a 10 sec pulse with an amplitude of 0.7 Pa followed by a steady fluid shear stress of 0.18 Pa for 5 min (Table1, Fig 2B).

Nitric Oxide determination

The conditioned medium was assayed for NO, which was measured as nitrite (NO_2^-) accumulation in the conditioned medium, using Griess reagent (1% sulfanilamide, 0.1% naphtylethelene-diamine-dihydrochloride, and 2.5 M H_3PO_4). The absorbance was measured at 540 nm. NO concentrations were

determined using a standard curve derived from known concentrations of $NaNO_2$ in non-conditioned culture medium. Data from separate experiments were collected and normalized with respect to static controls, and expressed as treatment-over-control (T/C) ratios, or as absolute values where indicated.

Statistics

Data were pooled from the results of at least five experiments for each fluid shear stress regime tested. The effects of treatment with fluid shear stress were analyzed with the non-parametric Wilcoxon rank sum test of the S-Plus 2000 package (release 1). Differences were considered significant at a p value <0.05.

RESULTS

Application of fluid flow for 5 min to the MC3T3-E1 cells did not result in visible changes in cell shape or alignment of the cells in the direction of the fluid flow (data not shown). No cells were removed by any of the fluid shear stress regimes, as assessed by visually inspecting the cultures before and after fluid shear stress treatment, and by measuring the total amount of DNA (control, 261 ± 19 ng; PFSS, 279 ± 27 ng; mean ± SEM of 6 experiments). We have measured the response to 10 and 15 min of steady and PFSS regimes (data not shown). We found that after 10 min of pulsating fluid shear stress, the NO response is still rate dependent, but only to a lesser extent; and that the response to a steady fluid shear stress, remains the same even after 10 min (8).

The NO response of MC3T3-E1 cells to a fluid shear stress with zero rate was first studied separately, by culturing cells under steady flow conditions for 5 min. Cells produced 6.67 ± 1.07 nmol after 5 min without flow treatment but with steady fluid shear stress, cells produced 11.65 ± 2.36 nmol NO after 5 min (values are mean ± SEM) (Fig. 3). Cells treated with the steady fluid shear

stress of 0.70 Pa gave a significant ($p<0.05$), almost two-fold NO release as compared to static control levels (Fig. 3).

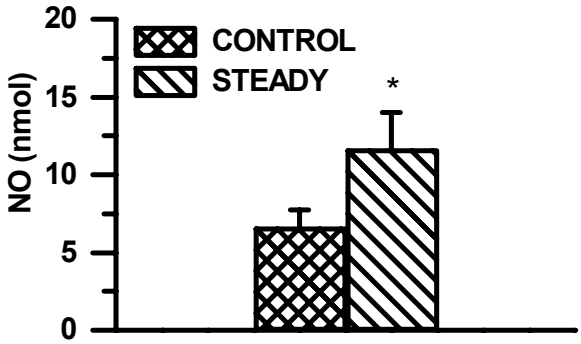

Figure 3. Effect of steady fluid shear stress on the NO production by MC3T3-E1 bone cells after 5 min. The absolute amount of NO produced by bone cells subjected to steady fluid shear stress (0.7 Pa), increased after 5 min as compared to static control cultures, which were not subjected to flow. Under steady fluid shear stress, cells produced 11.65 ± 2.36 nmol NO after 5 min. Cells produced 6.67 ± 1.07 nmol after 5 min without flow treatment (values are mean ± SEM). The response to steady shear stress increased 2-fold after 5 min. *significant effect of steady fluid shear stress, $p<0.05$.

To study the effect of the fluid shear stress frequency and amplitude on the NO response, cells were subjected to varying pulsating fluid shear stress (PFSS) regimes. NO production was between 3.63 ± 1.19 to 5.14 ± 1.26 nmol (mean ± SEM) per $2x10^5$ cells for cell cultures without fluid shear stress treatment. Treatment with 5 Hz low-amplitude PFSS (0.70 ± 0.31 Pa) did not affect NO production after 5 min (Fig. 4). Treatment with 9 Hz low-amplitude PFSS (0.70 ± 0.31 Pa) caused a rapid 3-fold increase of NO production after 5 min ($p<0.05$), as did treatment with 5 Hz high-amplitude PFSS (0.70 ± 0.70 Pa) after 5 min ($p<0.006$) (Fig. 4). Thus, bone cells showed increased NO production in response to PFSS with increased frequency or amplitude.

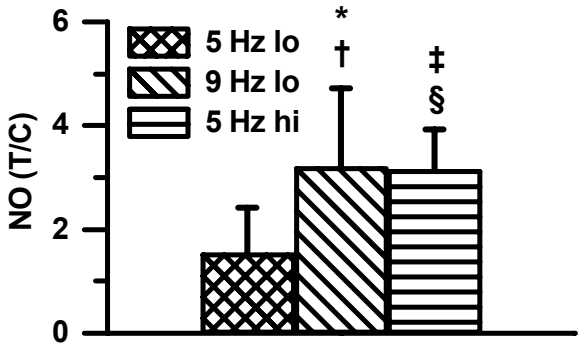

Figure 4. Effect of PFSS frequency and amplitude on the NO production by MC3T3-E1 bone cells after 5 min. NO production was between 3.63 ± 1.19 to 5.14 ± 1.26 nmol (mean ± SEM) per $2x10^5$ cells for all cell cultures without fluid shear stress treatment (static controls). Treatment with high- amplitude or frequency elicited higher NO response than low amplitude or frequency. Values are mean treatment-over-control ratios (T/C)±SEM. lo, low-amplitude (0.31 Pa); hi, high-amplitude (0.70 Pa). *, Significantly different from 1.0, $p<0.05$; †, significantly different from 5 Hz low-amplitude, $p<0.05$; ‡, significantly different from 1.0, $p<0.006$; §, significantly different from 5 Hz low-amplitude, $p<0.006$.

The effect of flow regimes on the NO response of bone cells, without an initial stress-kick was then studied by subjecting bone cells to PFSS with a 5 min pre-treatment phase (Fig. 2A, Fig. 5A). The initial stress-kick was avoided by slowly rising the fluid shear stress from 0 to 0.7 Pa for 3 min, and then inducing steady fluid shear stress for 2 min (the 5 min pre-treatment phase, Fig. 2A) before the application of the PFSS regimes for 5 min (Fig. 5A). Application of the 5 min pre-treatment phase as well as the subsequent application of PFSS did not affect the NO production of the cells (Fig. 5A). The effect of a steady fluid shear stress without an initial stress-kick at a shorter time point was studied by subjecting bone cells to a slow rise of shear stress from 0 to 0.18 Pa or 0.7 Pa for 1 min (the 1 min pre-treatment phase, Fig. 2B) before inducing a steady shear stress of 0.18 Pa or 0.7 Pa for 5 min,

respectively (Fig. 2A, Fig. 5B). NO production was between 2.54 ± 0.54 to 3.30 ± 1.01 nmol (mean ± SEM) per $2x10^5$ cells for cell cultures without fluid shear stress treatment (static controls for both pre-treatment and subsequent PFSS treatment). Application of the 1 min pre-treatment phase as well as the subsequent steady fluid shear stress also did not affect the NO production (Fig. 5B). To investigate the effect of a short duration stress-kick, the cells were subjected to a single pulse shear (10 sec) with an amplitude of 0.7 Pa followed by a 5 min steady shear stress at 0.18 Pa (Fig. 2C, Fig. 5B). Application of the single pulse shear stress as well as the subsequent steady shear stress at 0.18 Pa did not affect the NO production.

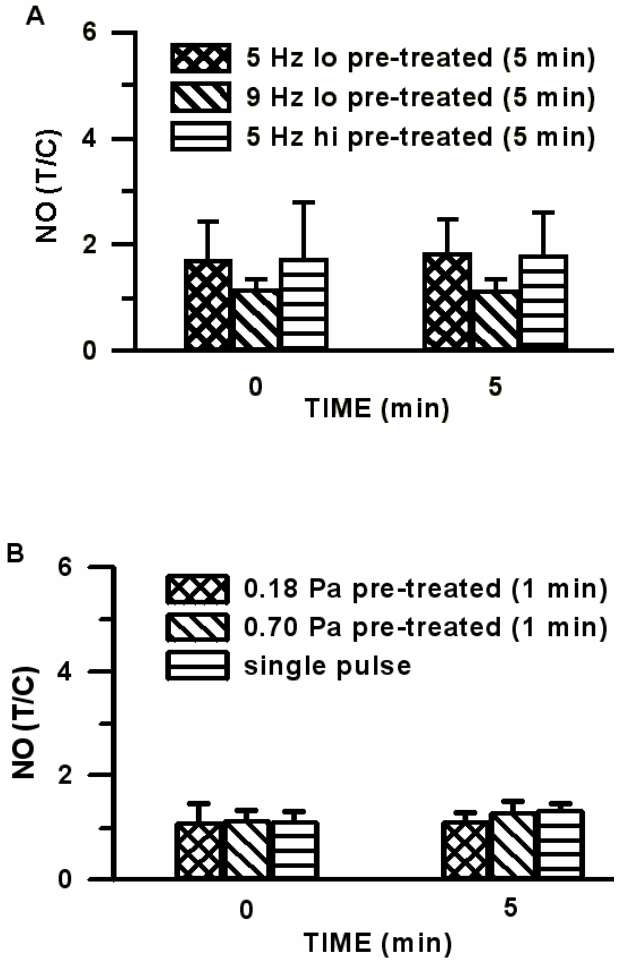

Figure 5. A. Nitric oxide production by bone cells in response to PFSS regimes without an initial stress-kick after 5 min. NO production was between 2.54 ± 0.54 to 3.30 ± 1.01 nmol (mean ± SEM) per $2x10^5$ cells for all cell cultures without fluid shear stress treatment (static controls for both pre-treatment and subsequent PFSS treatment). The pre-treatment phase is composed of a slowly rising flow from 0 to 8.30 ml/min to induce 0 to 0.7 Pa fluid shear stress for 3 min, followed by a steady flow at 8.30 ml/min to induce 0.7 Pa steady fluid shear stress for 2 min. The graph shows that application of the pre-treatment phase as well as the subsequent application of PFSS did not affect the NO production by bone cells. B. Nitric oxide production by bone cells in response to a single pulse shear stress. NO production without fluid shear stress treatment was similar as in A (static controls for both pre-treatment or pulse shear and the subsequent application of shear stress). The single pulse shear stress was applied with a 10s steady shear of 0.7 Pa, followed by a 5 min steady shear stress of 0.18 Pa. Application of the single pulse shear stress as well as the subsequent 0.18 Pa did not affect the NO production by bone cells. Values are mean treatment-over-control ratios (T/C)±SEM. lo, low-amplitude (0.31 Pa); hi, high-amplitude (0.70 Pa).

DISCUSSION

The present study suggests that the bone cell response to fluid shear stress is rate- dependent, but that an initial stress kick is required for the cells to respond. Increasing either the shear stress amplitude or frequency, without increasing the average stress, enhanced NO production by bone cells *in vitro*. Bone cells responded similarly to similar rates of fluid shear stress, despite different frequencies (5 Hz, 21.99 Pa-Hz or 9Hz, 17.53 Pa-Hz). Hence, the rate of fluid shear stress (i.e., $2\pi \times f \times \tau$) is proportional to the product of the fluid shear stress amplitude and frequency (8).

Our *in vitro* results support the notion that bone formation *in vivo* is stimulated by dynamic rather than static loads (40), and that low-magnitude, high-frequency mechanical stimuli may be as stimulatory as high-amplitude, low-frequency stimuli, provided that the cells are "kicked" in a pre-conditioned state. The steady, non-dynamic shear stress gave a zero shear rate, but also elicited a significant NO response. When the steady shear stress was applied, the cells experienced a sudden increase of fluid stress from 0 to 0.70 Pa in less than a second, after the pump was switched on. This suggests that bone cells responded significantly to an initial rapid stress increase. The pre-treatment phase, eliminating the initial stress-kick, strongly decreased the NO response of bone cells to fluid shear stress. The initial stress condition seems to define how bone cells respond to the ensuing fluid shear stress regime. This suggests that bone can be pre-conditioned to the environmental stress state by impact loading, as e.g. during exercise like jogging. Conversely, lack of stress-kicks may impair the sensitivity of bone cells to stress, contributing to an insufficient maintenance of bone quality. This might play a role in disuse osteoporosis induced by prolonged bed rest or microgravity, where impact loading conditions are absent. The finding that the bone cell's response to fluid shear stress is rate dependent provides an explanation why adaptive bone formation can occur despite the sporadic occurrence of high-amplitude strains in daily life (28).

The change in the sensitivity of bone cells to fluid shear stress in the absence of a stress-kick might be due to a cytoskeleton-mediated change in cellular mechanical properties. It has been shown that the ability of cells to respond to mechanical stimuli is mediated by its cytoskeleton (41), which is important for the fluid shear stress response of bone cells (38, 42). The cytoskeletal stiffness (ratio of stress to strain) was shown to increase in response to increasing mechanical stress (41). Furthermore, Pavalko and co-workers showed that MC3T3-E1 actin cytoskeleton rearranges in response to fluid flow stimulation (42). Therefore the transient pre-treatment phase to remove the initial stress-kick might have influenced bone cell cytoskeletal stiffness to make them less sensitive to the following fluid shear stress regime. Our results showed that the cells did not distinguish between a 1 min or 5 min pre-treatment phase (which removed the initial stress-kick) suggesting that the cellular sensitivity to stress might have been affected already within 1 min. However, the cells were also not affected by the application of a single pulse shear stress, i.e. a short duration stress-kick (10 sec, 0.7 Pa amplitude). Thus, the period between 10 sec and 1 min might be crucial for modulating the NO production by bone cells.

Interestingly, an initial stress-kick was shown to be essential for a stable response of bone cells. This corresponds to recent findings that low-frequency, high-amplitude loads and high-frequency, low-amplitude noise give a stronger response than the two signals alone (33). This might also indicate that bone cells have an initial activation barrier in terms of a loading threshold. The necessity of an initial stress kick for bone cells to respond to dynamic loading implies an essential non-linearity to the way bone cells respond to mechanical stress. This provides a cellular basis for stochastic resonance to occur in bone as a non-linear biological system (33). Based on our findings, we suggest that the history of stress pre-conditions the way bone cells respond to mechanical loading. Hence, the absence of high rate stresses might impair the mechanosensitivity of bone cells.

We conclude that NO production by bone cells subjected to fluid flow is dependent on the rate of fluid shear stress. The amplitude and frequency of stress as well as the initial stress conditions contribute to the flow-induced activation of bone cells. We hypothesize, that high rate stresses pre-condition the cells in such a way, that they become sensitive to mechanical loading.

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