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Quantification of Lacunar–Canalicular Interstitial Fluid Flow Through Computational Modeling of Fluorescence Recovery After Photobleaching

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Abstract

Skeletal adaptation to mechanical loading has been widely hypothesized to involve the stimulation of osteocytes by interstitial fluid flow (IFF). However, direct investigation of this hypothesis has been difficult due in large part to the inability to directly measure IFF velocities within the lacunar–canalicular system. Measurements of fluorescence recovery after photobleaching (FRAP) within individual lacunae could be used to quantify lacunar–canalicular IFF when combined with mathematical modeling. In this study, we used a computational transport model to characterize the relationship between flow frequency (0.5–10 Hz), peak flow velocity (0–300 $\mu\text{m/s}$), tracer diffusion coefficient (100–300 $\mu\text{m}^2/\text{s}$), and transport enhancement (i.e., $(k/k_0) - 1$, where k and k_0 are the transport rates in the presence/absence of flow) during lacunar FRAP investigations. We show that this relationship is well described by a simple power law with frequency-dependent coefficients, and is relatively insensitive to variations in lacunar geometry. Using this power law relationship, we estimated peak IFF velocities in hindlimb mice subjected to intramedullary pressurization using values of k and k_0 previously obtained from *ex vivo* lacunar FRAP investigations. Together, our findings suggest that skeletal adaptation in hindlimb suspended mice subjected to dynamic intramedullary pressure occurred in the presence of IFF at levels associated with physiological loading.

Keywords

Interstitial fluid flow; FRAP; Osteocyte; Mechanotransduction; Transport

INTRODUCTION

The capacity for bone to adapt its structure in response to mechanical forces has long been recognized. However, many of the physical, cellular, and molecular mechanisms underlying this process are not well understood. It has long been hypothesized that skeletal mechanotransduction may be mediated by the stimulation of osteocytes by interstitial fluid flow (IFF).²¹ In particular, both theoretical^{2,16,21,37} and experimental^{14,15,22} investigations have demonstrated that mechanical loading of bone induces movement of IFF within the interconnected lacunar–canalicular system (LCS) in which osteocytes reside. This IFF subjects osteocytes to a variety of potential stimuli including enhanced nutritional transport,²¹ streaming potentials,² and mechanical loads arising from fluid shear stresses on the cell surface³⁷ as well drag forces on the pericellular matrix.^{11,38} A number of experimental investigations support a role for IFF in mediating loading-induced adaptation, including *in vitro* studies demonstrating

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the capacity of osteocytes to sense and respond to physiological levels of fluid shear stress,^{1, 8,13,18,20,31,39} and several *in vivo* investigations demonstrating changes in osteocyte physiology in mechanically loaded bone.^{4–6,19,25} However, although these investigations provide indirect evidence that skeletal mechanotransduction may be mediated by IFF and the subsequent stimulation of osteocytes, more direct evidence has remained elusive,⁷ due in large part to the lack of ability to directly measure IFF velocities within the microscopic fluid spaces of the LCS.⁷

Recently, Wang and coworkers developed a novel technique for investigating solute transport within the LCS based on the measurement of fluorescence recovery after photobleaching (FRAP) within individual lacunae.³⁴ In this approach, a fluorescent tracer is vitally injected and allowed to equilibrate within the pericellular space of the LCS. A confocal microscope is used to photobleach a single lacuna on the periosteal surface, and the subsequent recovery of fluorescence due to infilling of unbleached tracer molecules from surrounding lacunae and canaliculi is monitored. Characteristic transport rates are quantified from the recovery curves. Su *et al.* extended this technique to investigate effects of knee loading on transport within the LCS, and demonstrated that enhanced solute transport due to loading-induced IFF could be detected by observing differences in transport rates in the presence and absence of mechanical loading.²⁸

Mathematical models could potentially be used to quantify loading-induced IFF velocities within the LCS by fitting numerical results to experimental data obtained from lacunar FRAP investigations. For example, Zhou and coworkers recently developed a mathematical transport model that simulated FRAP within a lacunar–canalicular unit in the presence of dynamic IFF,⁴¹ and subsequently used the model to investigate the effects of different tracer sizes and canalicular geometries in simulated FRAP experiments.⁴² By simulating FRAP over a wide range of flow profiles, a similar modeling approach could potentially be used to determine a relationship between fluorescence recovery transport rate, flow frequency, and peak flow velocity, allowing for quantification of lacunar–canalicular IFF from experimentally obtained lacunar FRAP transport rates in the presence/absence of flow. Loading-induced increases in lacunar FRAP transport rates have recently been characterized during intramedullary pressurization of the femur,¹⁷ knee loading,²⁸ and loading of the tibia.²² Since all of these loading modalities have been associated with skeletal adaptation, it would be highly informative to know the extent to which IFF was being generated within the LCS in these studies.

In this study, we used a computational transport model to determine an empirical relationship between flow frequency (0.5–10 Hz), peak flow velocity (0–300 $\mu\text{m/s}$), solute diffusion coefficient (100–300 $\mu\text{m}^2/\text{s}$), and transport enhancement (i.e., $(k/k_0) - 1$, where k and k_0 are the transport rates in the presence/absence of flow) that allows for quantification of peak IFF velocities within the LCS from transport rates experimentally obtained from lacunar FRAP investigations. We show that this relationship, which can be well described by a simple power law with frequency-dependent coefficients, is relatively insensitive to physiological variations in lacunar geometry and can be modified to take into account certain cases of non-uniform canalicular flow. As an application of our numerical results, we estimated peak IFF velocities in hindlimb suspended mice subjected to dynamic pressurization of the femoral intramedullary cavity using the power law relationship derived in this study and values of k and k_0 previously obtained from *ex vivo* lacunar FRAP investigations.¹⁷ Together, our findings suggest that skeletal adaptation to dynamic intramedullary pressure in mice subjected to hindlimb suspension occurred in the presence of IFF at levels associated with physiological loading.

METHODS

Model Geometry

We developed a one-dimensional transport model in order to model FRAP within a single lacuna. Our model builds on the model of Zhou *et al.*,^{41,42} which assumed uniform convective velocities in all canaliculi, by allowing for two different canaliculi domains, one of which allows for convective and diffusive transport (i.e., canaliculi which undergo flow), and the other of which only allows for diffusion (canaliculi which do not undergo flow). The model geometry consists of an ellipsoid (lacuna) with equatorial radius a and polar radius b coupled to two cylindrical channels of length d (canaliculi) on either end (Fig. 1). The dimensions of the channels and ellipsoid are such that the cross-sectional area profile mimics that of the pericellular fluid space across a lacunar–canaliculi unit, assuming that an osteocyte cell body and its processes partially fill the space within the lacuna and canaliculi, respectively. We assume there are N_{tot} canaliculi entering an individual lacuna, and a fraction φ ($0 < \varphi \leq 1$) of these canaliculi contribute to tracer transport into the lacuna. The parameter φ is a correction factor that accounts for photobleaching of tracer molecules within lacunae and canaliculi above and below the image plane,³⁴ and depends on the three-dimensional photobleaching profile and its orientation relative to the photobleached lacuna. In this case, the total number of contributing canaliculi is

$$N = \varphi N_{\text{tot}}. \quad (1)$$

Consider an idealized osteocyte extending N_{tot} cylindrical processes, each of radius r_i , into cylindrical canaliculi of radius r_o . The total annular cross-sectional area of all of the non-photobleached canaliculi that contribute to tracer transport into the lacuna is $N\pi(r_o^2 - r_i^2)$. Now consider the model geometry, in which there are two channels, one on either side of the lacuna. The cross-sectional area within each channel is calculated such that the combined cross-sectional area of the two channels is equal to the total cross-sectional area within all the canaliculi that contribute to tracer transport into the lacuna:

$$A(x) = 0.5N\pi(r_o^2 - r_i^2) \quad \text{for } a < |x| \leq d + a. \quad (2)$$

Note that the cross-sectional area of the channel is equivalent to the cross-sectional area of an annulus with outer and inner radii $\sqrt{0.5N}r_o$ and $\sqrt{0.5N}r_i$ (Fig. 1). Within the lacuna, the cross-sectional area is assumed to be an elliptical annulus with varying outer and inner minor radii a_o and a_i and outer and inner major radii b_o and b_i :

$$A(x) = \pi(a_o b_o - a_i b_i) \quad \text{for } |x| \leq a, \quad (3)$$

where

$$\begin{aligned} a_o &= \sqrt{0.5N}r_o + \sqrt{\left[a - \sqrt{0.5N}r_o\right]^2 \left[1 - (x^2/a^2)\right]} \\ b_o &= \sqrt{0.5N}r_o + \sqrt{\left[b - \sqrt{0.5N}r_o\right]^2 \left[1 - (x^2/a^2)\right]} \\ a_i &= \sqrt{0.5N}r_i + \sqrt{\left[a - \sqrt{0.5N}r_i - w\right]^2 \left[1 - (x^2/a^2)\right]} \\ b_i &= \sqrt{0.5N}r_i + \sqrt{\left[b - \sqrt{0.5N}r_i - w\right]^2 \left[1 - (x^2/a^2)\right]} \end{aligned} \quad (4)$$

and w is the peak fluid gap width between the lacunar wall and the osteocyte body. It is noteworthy that at $x = |a|$, the lacunar cross-sectional area is equal to that of each channel, $0.5N\pi(r_o^2 - r_i^2)$. In this geometry, flow occurs perpendicular to the polar axis of the lacuna (Fig. 1). Consider a long bone in which we decompose IFF within the LCS into three principal directions: radial, circumferential, and longitudinal. In this case, if we assume that the polar axes of periosteal lacunae in the diaphyses and/or metaphyses of murine long bones are oriented primarily in the longitudinal direction (as they are in the diaphyses of murine tibiae²⁶), then flow which is perpendicular to the lacunar polar axis (as in the model) can be considered to be occurring in the radial and/or circumferential directions.

Solute Conservation Equations and Fluid Velocities

Tracer transport into the photobleached lacuna is assumed to occur solely due to diffusion and/or convection via canaliculi connecting the photobleached lacuna with neighboring lacunae. The interstitial fluid in which the tracer molecules reside is assumed to be incompressible. We assume that transport within a fraction ε ($0 < \varepsilon \leq 1$) of the canaliculi occurs by diffusion and convection, while transport in the remaining fraction $(1 - \varepsilon)$ occurs solely by diffusion. Let $C_\varepsilon(x, t)$ and $C_{1-\varepsilon}(x, t)$ be the concentration profiles within these two sets of canaliculi, respectively. The solute conservation equations within the channels are

$$\frac{\partial C_\varepsilon}{\partial t} = D \frac{\partial^2 C_\varepsilon}{\partial x^2} - v \frac{\partial C_\varepsilon}{\partial x} \quad \text{for } a < |x| \leq d+a \quad (5)$$

and

$$\frac{\partial C_{1-\varepsilon}}{\partial t} = D \frac{\partial^2 C_{1-\varepsilon}}{\partial x^2} \quad \text{for } a < |x| \leq d+a \quad (6)$$

where D is the diffusion coefficient and v is the convective velocity. Within the lacuna, the cross-sectional area is spatially varying. Let $C(x, t)$ be the concentration profile within the lacuna. The solute conservation equation in this case is given by Zhou *et al.*⁴¹ as

$$A \frac{\partial C}{\partial t} = \frac{\partial}{\partial x} \left(AD \frac{\partial C}{\partial x} \right) - Av \frac{\partial C}{\partial x} \quad \text{for } |x| \leq a \quad (7)$$

where A is the area, and the flow rate (Av) is assumed to be spatially independent due to conservation of mass. The ellipsoid geometry of the lacuna is incorporated into the model through Eq. (7) and the spatially varying cross-sectional area $A(x)$ given by Eqs. (3) and (4). Equation (7) is the solute conservation equation in the case of one-dimensional convective and diffusive transport assuming a spatially varying cross-sectional area, and accounts for the effects of varying cross-sectional area on mass conservation as the cross-sectional area of the lacuna decreases from $x = 0$ (where the cross-sectional area is a maximum) to $|x| = a$ (where the cross-sectional area is equal to that of the channels). Within the channels, the velocity is assumed to be sinusoidal and is calculated as

$$v(x, t) = v_c \cos(2\pi ft) \quad \text{for } a < |x| \leq d+a \quad (8)$$

where v_c is the peak convective flow velocity within the canaliculi, and f is the frequency. Although oscillatory flow under physiological loading is not expected to be sinusoidal,¹² this waveform was chosen due to the widespread use of this profile in established models of *in vivo* skeletal loading,^{23,28–30,35} which are expected to be used to investigate the effects of mechanical loading on lacunar–canalicular solute transport rates in the future. Within the lacuna, the flow velocity is calculated by assuming that the lacunar flow rate is equal to the channel flow rate, as implied by conservation of mass:

$$v(x, t) = \frac{\varepsilon N \pi (r_o^2 - r_i^2) v_c \cos(2\pi f t)}{2A} \quad \text{for } |x| \leq a. \quad (9)$$

Initial and Boundary Conditions

The following conditions are imposed at the channel/lacunar interfaces:

$$C_\varepsilon = C_{1-\varepsilon} = C \quad \text{at } |x| = a \quad (10)$$

and

$$\frac{\partial C}{\partial t} = \varepsilon \frac{\partial C_\varepsilon}{\partial t} + (1 - \varepsilon) \frac{\partial C_{1-\varepsilon}}{\partial t} \quad \text{at } |x| = a. \quad (11)$$

Similar to previous models,^{34,41} surrounding lacunae are assumed to act as constant concentration sources, i.e.,

$$C_\varepsilon = C_{1-\varepsilon} = C_0 \quad \text{at } |x| = a + d, \quad (12)$$

where C_0 is a constant equal to the concentration of unbleached tracer within the source-lacunae. Immediately after photobleaching, the initial concentration is assumed to be linearly varying within the canaliculi and constant within the lacuna (Fig. 2), as described previously^{34,41}:

$$\begin{aligned} C_\varepsilon(x, 0) &= C_{1-\varepsilon}(x, 0) \\ &= (C_b - C_0) \frac{a - |x|}{d} + C_b \quad \text{for } a < |x| \leq d + a \\ C(x, 0) &= C_b \quad \text{for } |x| \leq a \end{aligned} \quad (13)$$

where C_b is a constant equal to the initial concentration of unbleached tracer within the photobleached lacuna.

Numerical Solution

To solve the above equations, a central difference scheme as described by Wang and Hutter³³ was implemented in the open source software package Octave (www.octave.org). The simulations were run with a uniform spatial discretization of $0.25 \mu\text{m}$ for a total simulation time of $0.6\text{--}2 \text{ s}$ with a time step of $1\text{--}10 \mu\text{s}$, depending on the frequency and diffusion coefficient used. For each simulation, the mean concentration within the lacuna $\bar{C}(t)$ was found, and the logarithmic recovery ratio $\ln[(C_0 - \bar{C}(t))/(C_0 - C_b)]$ computed.³⁴ The characteristic transport rate k was computed as the negative slope of the best fit line.⁴¹ For all simulations with non-

zero flow velocity, transport rates computed under pure diffusion (i.e., $v_c = 0$), denoted as k_0 , were also computed. For each simulation, the transport enhancement ζ was defined as $\zeta = (k/k_0) - 1$, where k_0 is the transport rate under identical simulation conditions except with $v_c = 0$ (i.e., the transport rate under pure diffusion). Unless noted otherwise, the following values were assumed: $N_{\text{tot}} = 54$,³⁴ $r_o = 130$ nm,⁴⁰ $r_i = 52$ nm,⁴⁰ $a = 4.0$ μm ,³⁴ $b = 8.6$ μm ,³⁴ $w = 2$ μm , $d = 26$ μm ,³⁴ $v_c = 100$ $\mu\text{m/s}$, $f = 5$ Hz, $C_b = 0.5$, $C_0 = 1$, $\phi = 0.2$,³⁴ and $\varepsilon = 1$.

Fitting of Data

Linear fitting of k_0 and nonlinear fitting of ζ was performed in the open source statistical environment *R* (<http://www.R-project.org>). For fitting of ζ , the percent error between the numerical data and the best nonlinear fit was calculated as

$$\% \text{ Error} = 100 \frac{\|\zeta_{\text{num}} - \zeta_{\text{fit}}\|_2}{\|\zeta_{\text{num}}\|_2} \quad (14)$$

where ζ_{num} is the transport enhancement obtained from the numerical simulation, ζ_{fit} is the transport enhancement obtained from the best fit, and $\|\zeta\|_2$ denotes the L2-norm of ζ .

RESULTS

Typical Solutions

A typical solution during a single flow oscillation can be seen in Fig. 2a, and typical logarithmic recovery ratios over time can be seen in Fig. 2b. For both zero and non-zero convective velocities, the logarithmic recovery ratios decrease linearly with time. However, there is an increase in the transport rate in the presence of flow, which is dependent on the fraction of canaliculi undergoing convective transport. The linear decrease in the logarithmic recovery ratios is consistent with numerical simulations,^{41,42} as well as experimental results obtained under a variety of loading conditions.^{17,22,28}

Effects of the Number of Contributing Canaliculi and Diffusion Coefficient on k_0

We characterized the behavior of our model solely under diffusion by finding k_0 assuming different values for the number of contributing canaliculi (i.e., $N = \phi N_{\text{tot}}$ for $\phi = 0.2-1$) and tracer diffusion coefficients ($D = 25-200$ $\mu\text{m}^2/\text{s}$). We found that k_0 could be fit as

$$k_0 = KND, \quad (15)$$

where K is a constant equal to 4.5×10^{-6} μm^{-2} ($R^2 = 0.9996$) (Fig. 3). The linear scaling of k_0 with N and D is consistent with previous analytical investigations using a source-sink model.³⁴ There was a ~7% difference between the value of K found in our studies and the value of $K = 4.2 \times 10^{-6}$ μm^{-2} obtained using the source-sink model of Wang *et al.*:

$$K = A_{\text{can}} / (V_{\text{lac}} d) \quad (16)$$

where

$$A_{\text{can}} = \pi(r_o^2 - r_i^2) \quad (17)$$

is the cross-sectional area of the canalicular fluid space, and

$$V_{\text{lac}} = \frac{4\pi}{3} [ab^2 - (a-w)(b-w)^2] \quad (18)$$

is the volume of the lacunar fluid space.³⁴ This indicates that the use of the analytical model of Wang *et al.* or the computational model in this study only modestly affects the calculation of diffusion coefficients from experimental FRAP data if identical lacunar–canalicular geometries and model parameters are assumed.

Effects of Canalicular and Lacunar Geometry on Transport Enhancement

We next investigated the effects of altering ϕ , w , a , and b on transport rates and enhancement. A value of $\phi = 0.22$ has been proposed for FRAP data obtained using a laser scanning confocal microscope,³⁴ but would likely be higher for data obtained under an imaging modality that had less out-of-plane photobleaching, such as two-photon microscopy (as in Su *et al.*²⁸). In our model, both k_0 and k increased substantially when we increased ϕ from 0.2 to 1 ($k_0 = 1.0 \times 10^{-2}$ to $4.7 \times 10^{-2} \text{ s}^{-1}$ and $k = 1.1 \times 10^{-2}$ to $5.2 \times 10^{-2} \text{ s}^{-1}$). However, transport enhancement ξ remained relatively constant (ranging from $\xi = 0.11$ – 0.12). These data are consistent with the findings of Zhou *et al.*, who found that ξ was relatively insensitive to the number of contributing canaliculi.⁴² Note that in the model, decreasing ϕ from 1 to 0.2 while keeping all other parameters constant is mathematically equivalent to setting $\phi = 1$ and decreasing r_o from 130 nm to $0.4 \times 130 = 52$ nm and r_i from 52 nm to $0.4 \times 52 = 21$ nm. This indicates that ξ may also be insensitive to anatomical variations in osteocyte process and canalicular diameter, although further investigation is necessary in this regard. When we increased the peak fluid gap width w from 0.2 to 5 μm , both k_0 and k decreased over an order of magnitude ($k_0 = 7.1 \times 10^{-2}$ to $7.2 \times 10^{-3} \text{ s}^{-1}$ and $k = 7.9 \times 10^{-2}$ to $8.1 \times 10^{-3} \text{ s}^{-1}$) (Fig. 4). This decrease occurred nonlinearly, with greater changes occurring for smaller values of w . In contrast, the transport enhancement ξ remained relatively constant ($\xi = 0.11$ – 0.12), varying less than 10% despite the fact that the peak fluid gap width spanned more than an order of magnitude. We next investigated the effects of altering the lacunar radii ($a, b = 5$ – $20 \mu\text{m}$; $w = 1 \mu\text{m}$ for all simulations) (Fig. 5). In this case, lacunar fluid volumes varied over an order of magnitude, and both k_0 and k varied substantially ($k_0 = 1.3 \times 10^{-2}$ to $2.7 \times 10^{-1} \text{ s}^{-1}$ and $k = 1.5 \times 10^{-2}$ to $3.1 \times 10^{-1} \text{ s}^{-1}$) over the range of radii tested. Transport rates increased with decreasing radii, with greater increases occurring with smaller radii. Again, the transport enhancement ξ was relatively constant over the range of radii simulated ($\xi = 0.09$ to 0.12).

Effects of Peak Convective Velocity, Frequency, and Diffusion Coefficient on Transport Enhancement

We next investigated the effects of altering values for the peak convective velocity ($v_c = 0$ – $300 \mu\text{m/s}$), flow frequency ($f = 0.5$ – 10 Hz), and diffusion coefficient ($D = 100$ – $300 \mu\text{m}^2/\text{s}$) on ξ (Fig. 6). Transport enhancement increased in a nonlinear manner with increasing peak convective velocities, decreasing frequencies of flow, and decreasing diffusion coefficients. We found that the following power law scaling was able to describe the simulation data relatively well:

$$\xi = \alpha(f) \psi^{\beta(f)} \quad (19)$$

where $\alpha(f)$ and $\beta(f)$ are unitless, frequency-dependent coefficients which are empirically determined, and ψ is a non-dimensional quantity,

$$\psi = \frac{v_c}{\sqrt{Df}}. \quad (20)$$

Table 1 contains the best fit values for $\alpha(f)$ and $\beta(f)$ for $f = 0.5, 1, 2, 5$, and 10 Hz, and the resulting errors between the simulation data and Eq. (19) when these best fit values were used. Note that Eq. (19) depends on D , the diffusion coefficient of the tracer within the LCS, which is generally not known *a priori*. However, we can combine Eq. (19) with Eq. (16) (the expression for K given by Wang *et al.*) and rearrange for v_c to give the peak convective velocity solely in terms of lacunar–canalicular geometry, transport rate under zero velocity (i.e., pure diffusion), and transport enhancement:

$$v_c = \left(\frac{\xi}{\alpha(f)} \right)^{1/\beta(f)} \sqrt{\frac{k_0 V_{\text{lac}} df}{NA_{\text{can}}}}. \quad (21)$$

Effects of the Number of Canalculi Undergoing Flow on Transport Enhancement

In the simulations described above, all canalculi were assumed to be experiencing flow at the same velocity (i.e., $\varepsilon = 1$). However, depending on the applied load, IFF may be generated primarily along a single direction.⁴¹ In this case, one would expect canalculi aligned parallel to this direction to experience high levels of flow, and those aligned perpendicular to this direction to experience low (or no) flow. Thus, we investigated the effect of altering the fraction of canalculi undergoing flow ($\varepsilon = 0.2$ – 1) on transport enhancement. We found that ξ increased with increasing values of ε , and that this increase was essentially linear (Fig. 7). This linear scaling did not depend on the diffusion coefficient ($D = 100$ – $300 \mu\text{m}^2/\text{s}$).

Quantification of IFF in Response to Intramedullary Pressurization

We recently developed a microfluidic system for generating dynamic intramedullary pressure and skeletal IFF in hindlimb suspended mice.¹⁷ In *ex vivo* lacunar FRAP investigations using disodium fluorescein as a tracer, we found that recovery rates were significantly increased by intramedullary pressurization.¹⁷ Here, we sought to estimate peak IFF velocities induced by intramedullary pressurization using the empirical relation between k_0 , ξ , and v_c obtained in this study (i.e., Eq. 21) and the values of k_0 and k experimentally obtained from our *ex vivo* lacunar FRAP investigations ($k_0 = 9.3 \times 10^{-3} \text{ s}^{-1}$ and $k = 11.7 \times 10^{-3} \text{ s}^{-1}$,¹⁷ resulting in a transport enhancement of $\xi = (k/k_0) - 1 = 0.26$). In particular, we substituted Eqs. (1), (17), and (18) into Eq. (21), and solved for v_c using the aforementioned values for k_0 and ξ and the following values: $\alpha(5 \text{ Hz}) = 1.5 \times 10^{-2}$, $\beta(5 \text{ Hz}) = 1.7$, $N_{\text{tot}} = 54$,³⁴ $\phi = 0.22$,³⁴ $w = 1 \mu\text{m}$,³⁶ $a = 3.7 \mu\text{m}$,³⁴ $b = 8.6 \mu\text{m}$,³⁴ $d = 26 \mu\text{m}$,³⁴ $r_o = 130 \text{ nm}$,⁴⁰ $r_i = 52 \text{ nm}$.⁴⁰ Following this process, we obtained a peak convective velocity of $v_c = 1.3 \times 10^2 \mu\text{m/s}$.

DISCUSSION

The measurement of FRAP within individual lacunae is a powerful approach to measure solute transport within the LCS. This technique may potentially allow for the quantification of

lacunar–canalicular fluid flow when combined with mathematical modeling. In this study, we used a computational transport model to determine transport enhancement during simulated lacunar FRAP experiments for various canalicular IFF peak velocities ($v_c = 0\text{--}300\ \mu\text{m/s}$), flow frequencies ($f = 0.5\text{--}10\ \text{Hz}$), and tracer diffusion coefficients ($D = 100\text{--}300\ \mu\text{m}^2/\text{s}$). The range of frequencies simulated in our studies were chosen based on frequencies previously used in animal models of skeletal mechanotransduction involving dynamic loading.^{3,10,17,22,28,35} The diffusion coefficients chosen reflect the prevalent use of relatively small tracers such as disodium fluorescein in lacunar FRAP studies carried out thus far.^{17,22,28} We found that the simulation data could be well described by a power law of the non-dimensional quantity ψ with frequency-dependent coefficients, resulting in 2% error or less for all frequencies simulated. This functional form was chosen for its simplicity; however, it is important to note that we did not find a consistent trend in the scaling exponent $\beta(f)$ with increasing frequency, suggesting that if a closed form solution exists, it likely takes on a more complex form than the power law proposed here.

We surmised that the utility of the numerical data and empirical relationship described above could be greatly enhanced if they could be shown to be relatively insensitive to variations in lacunar geometry associated with differences in species, anatomical site, and/or type of bone. For example, Zhou *et al.* demonstrated that transport enhancement is relatively insensitive to physiological variations in canalicular geometry (in particular, length and number), however the dependence of this parameter on lacunar geometry was not investigated. Thus, we characterized the dependence of transport enhancement on assumed dimensions of the lacuna and the pericellular fluid space. We found that ζ was relatively insensitive to variations in lacunar polar and equatorial radii. Previous investigations have shown that osteocyte lacunar size can vary substantially within the same bone, and is species- and bone type-dependent. In particular, Gray found that lacunar lengths could vary almost twofold within the same bone, and that there was an approximately 20% difference in the lacunar length in human and rat femurs.⁹ The range of lacunar polar and equatorial radii in our simulations spanned a range of values greater than these physiological variations. Thus, although Eq. (19) was established using fixed values of a and b , our findings suggest that this relationship will be relatively insensitive to the physiological variations in lacunar geometry described above.

We also found that transport enhancement was relatively insensitive to variations in fluid gap width, varying less than 10% over a range that spanned over an order of magnitude. Reported dimensions of the osteocyte cell body pericellular space can vary substantially depending on methodology. For example, using light microscopy, Wassermann and Yaeger³⁶ found relatively wide spaces ($2\text{--}3\ \mu\text{m}$) in fixed and demineralized bone, but much smaller spaces in sections of fresh frozen bone ($0.7\ \mu\text{m}$ on average). Investigations using electron micrographs of undemineralized sections revealed that the osteocyte cell body pericellular space could vary by an order of magnitude ($0.1\text{--}2.0\ \mu\text{m}$). Our simulations indicate that transport enhancement during FRAP investigations is minimally impacted by variations in lacunar fluid space width over this range. When considered in conjunction with our findings showing similar insensitivity regarding variations in lacunar size, as well as findings by Zhou *et al.* that transport enhancement is relatively constant over physiological variations in canalicular length and number, the collective findings of these studies suggest that transport enhancement is relatively insensitive to anatomical variations in lacunar–canalicular geometry, and that Eq. (19) may be valid for lacunar FRAP studies performed in a wide range of species, anatomical sites, and types of bone.

Previous models have demonstrated that loading-induced IFF can vary substantially in the radial direction relative to the circumferential direction in terms of both magnitude⁴¹ and temporal profile.²⁷ By allowing for two different canalicular domains, one of which allows for convective and diffusive transport (i.e., canaliculi which undergo flow), and the other of which

only allows for diffusion (i.e., canaliculi which do not undergo flow), our model allowed for the examination of a particular case of non-uniform flow in which canaliculi primarily oriented in a particular direction experience approximately the same flow, while canaliculi oriented perpendicular to this direction experience little (or no) flow. In this case, we found that there was a linear dependence of ζ on the fraction of canaliculi undergoing flow. The degree of heterogeneity in regards to canalicular fluid flow induced by various modalities of mechanical loading has not been well characterized. However, we can roughly estimate the number of canaliculi undergoing flow in response to a unidirectional pressure gradient by grouping canaliculi into three principal directions, and assuming that canaliculi aligned parallel to this pressure gradient all experience the same levels of flow, while the other canaliculi do not experience flow. In this case, the linear scaling of ζ with ε suggests that peak convective velocities calculated using Eq. (19) may be corrected for this particular case of non-uniform flow by multiplying velocities by a factor of ~ 3 .

We recently demonstrated that 3 min/day of dynamic intramedullary pressurization abrogated femoral bone loss in mice subjected to hindlimb suspension.¹⁷ In the same studies, we found that dynamic intramedullary pressurization also significantly increased FRAP within periosteal lacunae *ex vivo*.¹⁷ In assessing the role of lacunar–canalicular IFF in mediating skeletal mechanotransduction, it would be informative to know the levels of IFF that were generated in those studies, particularly since adaptation was found to occur in the virtual absence of matrix strain.¹⁷ Using the values of k_0 and ζ obtained from the *ex vivo* lacunar FRAP investigations and the power law relationship between v_c , k_0 , and ζ derived in this study (i.e., Eq. 21), we calculated a peak convective velocity of $v_c = 1.3 \times 10^2 \mu\text{m/s}$. This estimation of peak canalicular flow velocity is based on FRAP measurements at the periosteal surface, however it is likely that similar or even higher levels of flow occurred at the endosteal surface due to intramedullary pressurization.^{17,24} Interestingly, this level of IFF is similar to the canalicular fluid velocity of $\sim 100 \mu\text{m/s}$ predicted by Kufahl and Saha to occur in human bone during gait.¹⁶ It is also only slightly higher than the fluid velocity of $80 \mu\text{m/s}$ predicted by Zhou *et al.* to occur in mouse tibiae subjected to a oscillating axial load with a peak force of 3 N,⁴¹ which appears to generate tibial strains associated with ambulation and/or jumping in mice.³ Together, our findings indicate that skeletal adaptation in hindlimb suspended mice subjected to intramedullary pressurization occurred in the presence of IFF at levels associated with physiological loading. The use of transport models to quantify lacunar–canalicular flow from experimentally obtained FRAP data in mechanically loaded bone has been previously proposed,⁴¹ but to our knowledge this is the first time that this approach has been utilized in practice.

Several limitations need to be considered when interpreting our findings. First, the model assumes that transport into the photobleached lacuna occurs solely by transport from surrounding canaliculi, and neglects transport from unbleached tracer molecules located within the collagen-apatite porosity. It is unclear as to whether these pores form an interconnected pathway for fluid to flow. However recent evidence indicate that these pores do not form an interconnected porosity,³² suggesting they do not significantly contribute to transport of tracer into photobleached lacunae. Second, in our simulations, we assumed the solute velocity and the fluid velocity were equal, i.e., we neglected to account for reductions in solute velocity relative to the velocity of the fluid due to sieving by the pericellular matrix. Experimental data in bone regarding this phenomenon is not available.⁴¹ However, as this study focused on relatively small tracers ($D = 100\text{--}300 \mu\text{m}^2/\text{s}$), these reductions are not expected to be substantial, particularly for tracers with diffusion coefficients at the upper range of those simulated here. For example, Zhou *et al.* postulated that based on measurements of the endothelial glycocalyx, the velocity of a solute with a diffusion coefficient of $300 \mu\text{m}^2/\text{s}$ will be reduced by less than two percent due to sieving effects, assuming a similar matrix structure exists in bone.⁴¹ Third, in our model, we assumed a geometry mimicking radial/circumferential

flow. Our choice to focus our study on radial/circumferential flow rather than longitudinal flow was motivated by (a) the expected future use of these studies in elucidating levels of lacunar–canalicular IFF in animal models of long bone bending, and (b) mathematical models of long bone bending predicting levels of longitudinal IFF to be an order of magnitude lower than that in the radial and circumferential directions.²⁷ The expected levels of longitudinal flow in response to intramedullary pressurization have not been well investigated. However, it is noteworthy that computational models of turkey ulnae subject to intramedullary pressurization predict significant radial IFF, but not circumferential IFF, at the mid-diaphysis.²⁴ Fourth, the power law relationships derived in this study were determined assuming a sinusoidal flow profile; we did not investigate other waveforms. However, Zhou *et al.* found that recovery rates in the presence of flow are governed in large part by the peak displacement that the solute front travels during each loading cycle.⁴¹ In this case, we expect that the numerical results obtained in this study are applicable to lacunar FRAP data obtained under non-sinusoidal flow as long as the peak displacements of the solute front during each oscillation are similar to those occurring under sinusoidal flow. Finally, it is important to note that the experimental FRAP data utilized in this study were obtained *ex vivo* in harvested femurs, and it is possible that the lacunar–canalicular flow at the periosteal surface was altered by this harvesting procedure, and does not accurately reflect the flow that occurs *in vivo*. Several precautions were taken in order to minimize this possibility, including performing all imaging studies within 1 h of animal sacrifice, and leaving the periosteum intact.¹⁷ However, further investigations are necessary in order to determine differences of loading-induced enhancements in solute transport that occur *ex vivo* vs. those that occur *in vivo*.

In summary, we used a computational transport model to establish an empirical relationship between flow frequency, peak flow velocity, and transport enhancement, and showed that this relationship is expected to be relatively insensitive to physiological variations in lacunar geometry. We believe our findings will be of great use in examining the role of lacunar–canalicular IFF in mediating skeletal mechanotransduction in future investigations by allowing for the quantification of peak IFF levels from lacunar FRAP transport rates.

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References

- Cheng B, Zhao S, Luo J, Sprague E, Bonewald LF, Jiang JX. Expression of functional gap junctions and regulation by fluid flow in osteocyte-like MLO-Y4 cells. *J Bone Miner Res* 2001;16:249–259. [PubMed: 11204425]
- Cowin SC, Weinbaum S, Zeng Y. A case for bone canaliculi as the anatomical site of strain generated potentials. *J Biomech* 1995;28:1281–1297. [PubMed: 8522542]
- De Souza RL, Matsuura M, Eckstein F, Rawlinson SCF, Lanyon LE, Pitsillides AA. Noninvasive axial loading of mouse tibiae increases cortical bone formation and modifies trabecular organization: a new model to study cortical and cancellous compartments in a single loaded element. *Bone* 2005;37:810–818. [PubMed: 16198164]
- Dodd JS, Raleigh JA, Gross TS. Osteocyte hypoxia: a novel mechanotransduction pathway. *Am J Physiol Cell Physiol* 1999;277:C598–C602.
- Dodds RA, Ali N, Pead MJ, Lanyon LE. Early loading-related changes in the activity of glucose 6-phosphate dehydrogenase and alkaline phosphatase in osteocytes and periosteal osteoblasts in rat fibulae *in vivo*. *J Bone Miner Res* 1993;8:261–267. [PubMed: 8456583]
- Forwood MR, Kelly WL, Worth NF. Localisation of prostaglandin endoperoxide H synthase (PGHS)-1 and PGHS-2 in bone following mechanical loading *in vivo*. *Anat Rec* 1998;252:580–586. [PubMed: 9845208]

7. Fritton SP, Weinbaum S. Fluid and solute transport in bone: flow-induced mechanotransduction. *Annu Rev Fluid Mech* 2009;41:341–374.
8. Genetos DC, Kephart CJ, Zhang Y, Yellowley CE, Donahue HJ. Oscillating fluid flow activation of gap junction hemichannels induces ATP release from MLO-Y4 osteocytes. *J Cell Physiol* 2007;212:207–214. [PubMed: 17301958]
9. Gray DJ. Length of lacunae and number of canaliculi in bones of several mammals. *Anat Rec* 1941;81:163–169.
10. Gross TS, Srinivasan S, Liu CC, Clemens TL, Bain SD. Noninvasive loading of the murine tibia: an in vivo model for the study of mechanotransduction. *J Bone Miner Res* 2002;17:493–501. [PubMed: 11874240]
11. Han Y, Cowin SC, Schaffler MB, Weinbaum S. Mechanotransduction and strain amplification in osteocyte cell processes. *Proc Natl Acad Sci USA* 2004;101:16689–16694. [PubMed: 15539460]
12. Jacobs CR, Yellowley CE, Davis BR, Zhou Z, Cimbala JM, Donahue HJ. Differential effect of steady versus oscillating flow on bone cells. *J Biomech* 1998;31:969–976. [PubMed: 9880053]
13. Klein-Nulend J, Semeins CM, Ajubi NE, Nijweide PJ, Burger EH. Pulsating fluid flow increases nitric oxide (NO) synthesis by osteocytes but not periosteal fibroblasts—correlation with prostaglandin upregulation. *Biochem Biophys Res Commun* 1995;217:640–648. [PubMed: 7503746]
14. Knothe Tate ML, Knothe U. An ex vivo model to study transport processes and fluid flow in loaded bone. *J Biomech* 2000;33:247–254. [PubMed: 10653041]
15. Knothe Tate ML, Niederer P, Knothe U. In vivo tracer transport through the lacunocanalicular system of rat bone in an environment devoid of mechanical loading. *Bone* 1998;22:107–117. [PubMed: 9477233]
16. Kufahl RH, Saha S. A theoretical model for stress-generated fluid flow in the canaliculi-lacunae network in bone tissue. *J Biomech* 1990;23:171–180. [PubMed: 2312521]
17. Kwon RY, Meays DR, Tang WJ, Frangos JA. Microfluidic enhancement of intramedullary pressure increases interstitial fluid flow and inhibits bone loss in hindlimb suspended mice. *J Bone Miner Res*. 2010 Epub ahead of print.
18. Kwon RY, Temiyasathit S, Tummala P, Quah CC, Jacobs CR. Primary cilium-dependent mechanosensing is mediated by adenylyl cyclase 6 and cyclic AMP in bone cells. *FASEB J*. 2010 Epub ahead of print.
19. Lean JM, Jagger CJ, Chambers TJ, Chow JW. Increased insulin-like growth factor I mRNA expression in rat osteocytes in response to mechanical stimulation. *Am J Physiol Endocrinol Metab* 1995;268:E318–E327.
20. Malone AM, Anderson CT, Tummala P, Kwon RY, Johnston TR, Stearns T, Jacobs CR. Primary cilia mediate mechanosensing in bone cells by a calcium-independent mechanism. *Proc Natl Acad Sci USA* 2007;104:13325–13330. [PubMed: 17673554]
21. Piekarski K, Munro M. Transport mechanism operating between blood supply and osteocytes in long bones. *Nature* 1977;269:80–82. [PubMed: 895891]
22. Price C, Li W, Wang L. Direct measurement of load-induced solute transport within the lacunar–canalicular system of bone. *Trans Orthop Res Soc* 2010;35:0454.
23. Qin YX, Kaplan T, Saldanha A, Rubin C. Fluid pressure gradients, arising from oscillations in intramedullary pressure, is correlated with the formation of bone and inhibition of intracortical porosity. *J Biomech* 2003;36:1427–1437. [PubMed: 14499292]
24. Qin, YX.; McLeod, K.; Rubin, CT. Intramedullary pressure induced fluid flow in bone. *Proceedings of the First Joint BMES/EMBS Conference*; 1999. p. 490
25. Robling AG, Niziolek PJ, Baldridge LA, Condon KW, Allen MR, Alam I, Mantila SM, Gluhak-Heinrich J, Bellido TM, Harris SE, Turner CH. Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. *J Biol Chem* 2008;283:5866–5875. [PubMed: 18089564]
26. Schneider P, Stauber M, Voide R, Stampanoni M, Donahue LR, Müller R. Ultrastructural properties in cortical bone vary greatly in two inbred strains of mice as assessed by synchrotron light based micro- and nano-ct. *J Bone Miner Res* 2007;22:1557–1570. [PubMed: 17605631]
27. Srinivasan S, Gross TS. Canalicular fluid flow induced by bending of a long bone. *Med Eng Phys* 2000;22:127–133. [PubMed: 10854966]

28. Su M, Jiang H, Zhang P, Liu Y, Wang E, Hsu A, Yokota H. Knee-loading modality drives molecular transport in mouse femur. *Ann Biomed Eng* 2006;34:1600–1606. [PubMed: 17029032]
29. Torrance AG, Mosley JR, Suswillo RF, Lanyon LE. Noninvasive loading of the rat ulna in vivo induces a strain-related modeling response uncomplicated by trauma or periosteal pressure. *Calcif Tissue Int* 1994;54:241–247. [PubMed: 8055374]
30. Turner CH, Akhter MP, Raab DM, Kimmel DB, Recker RR. A noninvasive, in vivo model for studying strain adaptive bone modeling. *Bone* 1991;12:73–79. [PubMed: 2064843]
31. Vezeridis PS, Semeins CM, Chen Q, Klein-Nulend J. Osteocytes subjected to pulsating fluid flow regulate osteoblast proliferation and differentiation. *Biochem Biophys Res Commun* 2006;348:1082–1088. [PubMed: 16904067]
32. Wang L, Ciani C, Doty SB, Fritton SP. Delineating bone's interstitial fluid pathway in vivo. *Bone* 2004;34:499–509. [PubMed: 15003797]
33. Wang Y, Hutter K. Comparisons of numerical methods with respect to convectively dominated problems. *Int J Numer Methods Fluids* 2001;37:721–745.
34. Wang L, Wang Y, Han Y, Henderson SC, Majeska RJ, Weinbaum S, Schaffler MB. In situ measurement of solute transport in the bone lacunar–canalicular system. *Proc Natl Acad Sci USA* 2005;102:11911–11916. [PubMed: 16087872]
35. Warden SJ, Turner CH. Mechanotransduction in cortical bone is most efficient at loading frequencies of 5–10 Hz. *Bone* 2004;34:261–270. [PubMed: 14962804]
36. Wasserman F, Yaeger JA. Fine structure of the osteocyte capsule and of the wall of the lacunae in bone. *Z Zellforsch* 1965;67:636–652.
37. Weinbaum S, Cowin SC, Zeng Y. A model for the excitation of osteocytes by mechanical loading-induced bone fluid shear stresses. *J Biomech* 1994;27:339–360. [PubMed: 8051194]
38. You L, Cowin SC, Schaffler MB, Weinbaum S. A model for strain amplification in the actin cytoskeleton of osteocytes due to fluid drag on pericellular matrix. *J Biomech* 2001;34:1375–1386. [PubMed: 11672712]
39. You L, Temiyasathit S, Lee P, Kim CH, Tummala P, Yao W, Kingery W, Malone AM, Kwon RY, Jacobs CR. Osteocytes as mechanosensors in the inhibition of bone resorption due to mechanical loading. *Bone* 2008;42:172–179. [PubMed: 17997378]
40. You L, Weinbaum S, Cowin SC, Schaffler MB. Ultrastructure of the osteocyte process and its pericellular matrix. *Anat Rec Part A* 2004;278:505–513.
41. Zhou X, Novotny JE, Wang L. Modeling fluorescence recovery after photobleaching in loaded bone: potential applications in measuring fluid and solute transport in the osteocytic lacunar–canalicular system. *Ann Biomed Eng* 2008;36:1961–1977. [PubMed: 18810639]
42. Zhou X, Novotny JE, Wang L. Anatomic variations of the lacunar–canalicular system influence solute transport in bone. *Bone* 2009;45:704–710. [PubMed: 19576310]

FIGURE 1.

Schematic depicting the model geometry. The interstitial fluid space is shown in gray. The lacuna is assumed to be an ellipsoid with equatorial radius a (the radii in the x and z directions are identical) and polar radius b . The fluid space within the lacuna is assumed to be the pericellular space between the lacunar wall and the surface of the osteocyte cell body, with a peak gap width of w in the y and z directions at $x = 0$. The lacuna is coupled to two channels (canaliculi) of length d and radius $\sqrt{0.5N}r_o$ inhabited by osteocyte processes of radius $\sqrt{0.5N}r_i$, where N is the total number of unbleached canaliculi, and r_o and r_i are the radii of an individual canaliculus and osteocyte process. The fluid space within each channel is assumed to be the annular fluid volume between the channel wall and the surface of the osteocyte process. Fluid flow is assumed to occur perpendicular to the lacunar polar axis.

FIGURE 2.

Typical model solutions. (a) Evolution of concentrations within the canaliculi experiencing flow (C_e), canaliculi not experiencing flow (C_{1-e}), and lacuna (C) during a single flow oscillation from $t = 0$ – 0.2 s ($f = 5$ Hz, $v_c = 100 \mu\text{m/s}$, $\epsilon = 0.5$). Upon photobleaching ($t = 0$ s), the initial concentration is assumed to be linearly varying within the channels (from $C_0 = 1$ to $C_b = 0.5$) and constant within the lacuna ($C_b = 0.5$). Oscillatory fluid flow (initially in the $+x$ direction) is imposed immediately following photobleaching. The concentration profile in the canaliculi experiencing flow (C_e), but not in the canaliculi not experiencing flow (C_{e-1}), is visibly influenced by the imposed flow. Note that the influence of flow on the concentration profile within the lacuna (C) cannot be readily seen due to the substantially lower convective velocities in the lacuna relative to the canaliculi. (b) Logarithmic recovery ratios as a function of time in the absence of flow ($v_c = 0$, top line) or in the presence of oscillatory flow with $v_c = 100 \mu\text{m/s}$ and $\epsilon = 0.5$ (middle line) or $\epsilon = 1$ (bottom line). Transport rates are calculated as the negative slope of the line of best fit. Flow increases the transport rate in a manner that increases with the fraction of canaliculi undergoing flow.

FIGURE 3.

k_0 in the absence of flow for different diffusion coefficients ($D = 25\text{--}200\ \mu\text{m}^2/\text{s}$) and numbers of contributing canaliculi ($N = \phi N_{\text{tot}}$ for $\phi = 0.2\text{--}1$, $N_{\text{tot}} = 54$). We found that k_0 could be well fit as $k_0 = KND$, where $K = 4.5 \times 10^{-6}\ \mu\text{m}^{-2}$ ($R_2 = 0.9996$).

FIGURE 4.

Transport rates k_0 and k and transport enhancement ξ as a function of lacunar peak fluid gap width w . Both k_0 and k decreased over an order of magnitude over the range of w tested ($w = 0.2$ – $5 \mu\text{m}$), however, ξ remained relatively constant over this same range ($\xi = 0.11$ – 0.12).

FIGURE 5.

Transport rates k_0 and k and transport enhancement ζ as a function of the lacunar equatorial radius a and polar radius b . Both k_0 and k varied over an order of magnitude over the range of values tested ($a, b = 5\text{--}20\ \mu\text{m}$), however, ζ was relatively constant over the same range ($\zeta = 0.09\text{--}0.12$).

FIGURE 6.

Transport enhancement ζ as a function of peak convective velocity v_c , flow frequency f , and diffusion coefficient D . The top row shows ζ obtained from simulations, while the bottom row shows ζ obtained from a power law of the form $\zeta = \alpha(f)\psi^{\beta(f)}$, where $\psi = v_c(Df)^{-0.5}$ is a non-dimensional quantity, and $\alpha(f)$ and $\beta(f)$ are frequency-dependent, empirically determined coefficients.

FIGURE 7.

Transport enhancement ξ as a function of ε , the fraction of canaliculi experiencing flow, and tracer diffusion coefficient D . Transport enhancement linearly increased with ε . The linear scaling did not depend on the value of D .

TABLE 1

Best fit values for $\alpha(f)$ and $\beta(f)$, and the % error between simulation data and the best fit power law for each frequency.

Frequency f (Hz)	α	β	Error (%)
0.5	4.8×10^{-2}	1.6	1.4
1	4.2×10^{-2}	1.8	0.5
2	2.7×10^{-2}	2.0	0.2
5	1.5×10^{-2}	1.7	0.9
10	1.3×10^{-2}	1.7	0.3