7.2.5 Response of the chondrocyte to mechanical stimuli

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Introduction

Articular cartilage functions as a weight-bearing, wear-resistant material in synovial joints. Cartilage is subjected to a wide range of static and dynamic loading conditions, with peak stress amplitudes reaching 10–20 MPa (100–200 atmospheres) during activities such as stair climbing. The ability of cartilage to withstand compressive, tensile, and shear forces depends critically on the composition and structural integrity of its extracellular matrix (ECM). The maintenance of a functionally intact ECM requires the chondrocyte-mediated synthesis, assembly, and degradation of proteoglycans, collagens, non-collagenous proteins and glycoproteins, and other matrix molecules. The regulation of these metabolic processes in vivo appears to involve a combination of cell biological and physical mechanisms.

Clinical observations and animal studies in vivo suggest that joint loading and motion can induce a wide range of metabolic responses in cartilage. Immobilization or reduced loading can cause profound decreases in aggrecan synthesis and content, and a resultant softening of the tissue. In contrast, aggrecan concentration is often higher in areas of habitually loaded cartilage, and can be further increased by dynamic loading or remodeling of a joint, with concomitant restoration of biomechanical properties. More severe static, impact, or strenuous exercise loading can cause cartilage degradation with osteoarthritic changes. Thus, while some degree of normal joint loading appears to promote structural adaptation, abnormal mechanical forces predispose cartilage to degeneration.

The cell biological and biophysical transduction mechanisms by which chondrocytes respond to mechanical stimuli are not fully understood and are difficult to identify in vivo. Complexities include quantifying the biomechanics of loading, and distinguishing between direct effects of loading on cartilage metabolism and indirect (for example, loading-induced systemic) effects. An understanding of the cellular transduction mechanisms is further complicated by the important and complex role of the ECM and chondrocyte-ECM interactions. Thus, the use of isolated chondrocytes devoid of matrix may not be appropriate for testing hypotheses on mechanisms; conclusions would have to be interpreted with caution. As a result, cartilage explant systems and chondrocyte/gel-substrate culture systems have become increasingly important in the study of chondrocyte response to loading, since chondrocyte phenotypic expression and cell-matrix structure is preserved in these model systems.

Many physical phenomena which occur in cartilage during loading in vivo have been identified and quantified in vitro: compression of cartilage results in deformation of cells and extracellular matrix, hydrostatic pressure gradients, fluid flow, streaming potentials and currents, and physicochemical changes including altered matrix water content, fixed charge density, mobile ion concentrations, and osmotic pressure. Any of these mechanical, chemical, or electrical phenomena may modulate matrix metabolism (Fig. 7.23). An understanding of the spatial distribution of these forces and flows within cartilage, during compression, has been aided by the development of theoretical models for the mechanical, physicochemical, and electromechanical behavior of cartilage. Such models can provide a useful framework for correlating the spatial distributions of biosynthesis and physical stimuli that occur within cartilage explants during compression. Below, we review the results of studies designed to quantify the nature and kinetics of chondrocyte metabolic response to known mechanical stimuli, and then highlight recent in vitro studies designed to explore mechanotransduction mechanisms.
Effects of static / dynamic compression on matrix synthesis

Geometrically defined cartilage explants can be maintained in vitro in a stable and controlled biochemical and physical environment, suitable for testing the effects of mechanical stimuli on biosynthesis. Calf and adult bovine cartilage explants have been most commonly used; they can attain steady-state levels of aggregan synthesis, aggregation with hyaluronan and link protein, and turnover, suitable for studying perturbations caused by applied mechanical stimuli. Investigators have also used human, canine, and other tissue sources.

The application of mechanical compression directly to cartilage explants, using a range of amplitudes and frequencies, has been motivated by the physiologically relevant loading parameters used in animal studies. The qualitative trends seen in these animal studies have been substantiated and amplified using explant models: (1) static compression has been shown to significantly inhibit biosynthesis of proteoglycans and proteins, while (2) dynamic compression can markedly stimulate matrix production. The response to dynamic compression, however, depends on compression frequency. For example, biosynthesis in 3 mm diameter explants was not affected by low strain amplitude (1-4 per cent) unconfined compression at low frequency (<0.001 Hz), while aggregan and protein synthesis in these same explants were stimulated by compression at higher frequencies (0.01-1 Hz) (Fig. 7.24(a)). Chondrocytes cultured in agarose also exhibited inhibition of synthesis in response to static compression and stimulation of synthesis under 0.01-1 Hz dynamic compression, but only after the cells had synthesized and deposited de novo a dense extracellular matrix.

Several physical mechanisms (that is, the physical and chemical responses to these static and dynamic compression regimes) are postulated. Static compression has been shown to reduce the rate of transport of macromolecules, due to reduced average ECM pore size; change local ion concentrations, including pH in the pericellular matrix, via the Donnan effect, and alter cell and nucleus structure. Dynamic compression can additionally superimpose fluid flows, pressure gradients, and streaming currents or potentials. In vitro explant systems have the potential to be quantitative and specific in relating mechanical and biological parameters; selected examples are described in more detail below.

Kinetics of proteoglycan and protein biosynthetic response to compression

Measurements of the rate at which chondrocytes can sense and respond to mechanical stimuli can give insight regarding intracellular biosynthetic pathways and extracellular processing times. Previous studies have revealed that the inhibition of biosynthesis during static compression can occur as rapidly as one hour after application of compression. Recovery of biosynthesis after release of static compression can be much slower, and depends on the duration and amplitude of the static compression prior to release. After a two-hour static compression followed by release, aggregan synthesis recovered fully in another two hours. In contrast, after release of a 12-hour, 50 per cent static compression, 60 hours were necessary for biosynthesis to return to free-swelling levels. The recovery of synthesis of link protein, fibronectin, and total protein synthesis occurred more rapidly (also see below). These data suggest that cellular processes may be adapting to their new environment with increasing durations of static compression; protein synthesis may be initially blocked. Over time, the concentration of enzymes necessary for glycosylation may decrease, and hence, the longer the time necessary for recovery of aggregan synthesis with increased duration of compression. Compression-induced changes in cell and matrix morphology may also become less reversible with increased duration of compression.

Regarding intracellular processing, the intracellular aggregan core protein pool size was observed to decrease by 50 per cent in response to a 25 per cent static compression, whereas the intracellular rate of processing of core protein into proteoglycan was unaffected by compression. In contrast, four hours after release of a 50 per cent static compression, core protein pool size remained significantly decreased, and the rate of intracellular processing into proteoglycan was two-fold slower. Extracellular processing of newly-synthesized aggregan into proteoglycan aggregates has also been found to be sensitive to compression: the extracellular conversion of aggregan to a form capable of binding with hyaluronan was delayed by static
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forces and flows during dynamic compression: role of specific mechanical stimuli

To explore the effect of the dynamic component of compression on biosynthesis, Sah et al.42 and Kim et al.49 applied small amplitude, cyclic, unconfined compressions to cartilage disks, superimposed on a static offset compression. Experimental and control explants were subjected to the same level of static offset compression. Since low amplitude dynamic compression did not significantly alter the average water content or fixed charge density of the cartilage disks, there were negligible changes in the physicochemical status of the tissue. In this manner, the effects of oscillatory deformation of cells and matrix, fluid flow, streaming potentials, and hydrostatic pressure could be explored separately from physicochemical stimuli. The profiles of fluid velocity, streaming potential, and hydrostatic pressure, induced within cartilage by dynamic compression, will depend on the frequency of loading, specimen geometry, and the experimental loading configuration. At higher frequencies (for example, 1 Hz), there may be very little fluid exudation imbibition during each loading cycle, with elastic-like matrix deformation. In contrast, at lower frequencies, fluid flow within the tissue may be much more significant during each cycle.

In these experiments42,49, plane parallel, cylindrical disk specimens were compressed between fluid-impermeable platens used to simplify the loading configuration. However, even for this simple geometry, the spatial profiles of fluid velocity, and soon, are highly non-uniform within each disk. Theoretical analyses44,60 show that at higher-loading frequencies, the hydrostatic pressure within the ECM is highest in the central region of the cartilage disk (Fig. 7.24(b)); however, the fluid velocity and streaming potential fields are highest near the radial periphery of the disk.

Kim et al.24 took advantage of these non-uniform profiles and hypothesized that spatially non-uniform mechanical stimuli would produce a corresponding spatially non-uniform biosynthetic response. This would enable a direct experimental test of the relative importance of oscillatory fluid flow, hydrostatic pressure, streaming potential, and cell deformation in modulating chondrocyte metabolism during dynamic compression, via measurement of the frequency dependence and the spatial (radial) distribution of newly-synthesized matrix macromolecules within the cartilage explant disks44,49.

After dynamic compression of the 3 mm disks, the center 2-mm core of each disk was removed and the core and outer ring were analyzed separately for radiolabel incorporation. While compression at frequencies between 0.002 and 0.01 Hz caused a stimulation of biosynthesis that was distributed throughout the core and outer ring of the disk, compression at 0.1 Hz caused a stimulation that was confined mainly to the outer ring (Fig. 7.24(c)). (These radially dependent biosynthetic profiles were further confirmed by quantitative autoradiography.77) These distributions in incorporation were then compared to theoretical estimates of the radial distribution of forces and flows within the matrix44,49 (Fig. 7.24(b)). The observed biosynthetic patterns (Fig. 7.24(c)) most closely matched the radial dependence of fluid flow and its associated streaming potential, and cell deformation. Thus, the stimulation of chondrocyte biosynthesis by dynamic mechanical compression appeared associated mainly with changes in fluid flow, flow-induced streaming potentials, and/or cell shape, which were greatest near the disk periphery at higher frequencies (Fig. 7.24(b)), while hydrostatic pressure was probably less important.

Nevertheless, the slight biosynthetic stimulation in the central region, together with measured peak dynamic loads of about 0.5–1 MPa at the higher frequencies would be consistent with the possibility that hydrostatic pressure can stimulate biosynthesis. With increasing frequency (strain rates), dynamic compression of cartilage would induce increasing levels of hydrostatic pressure within the tissue. The effect of hydrostatic pressure on cartilage metabolism could be studied via mechanical compression of cartilage (as above), or more directly by pressurizing the fluid in a vessel containing chondrocytes or cartilage explants. The effects of hydrostatic pressure on chondrocyte activity in embryonic chicken, fetal mouse, bovine and human cartilage, and in rat chondrosarcoma cells have been reported.38-63 In general, static or low frequency hydrostatic pressure caused a decrease in GAG synthesis, while higher frequency hydrostatic loading caused
an increase in GAG synthesis. However, the effective threshold pressure for increased synthesis varied widely. In chick embryonic chondrocytes and fetal mouse cartilaginous long bone rudiments, cyclic pressures as low as 13 kPa\textsuperscript{41,62,64} elicited a stimulatory response, while pressures on the order of 5 MPa\textsuperscript{44-60} were needed to induce similar increases in matrix synthesis in bovine and human articular cartilage.

**Compression-induced fluid flow and solute transport**

Although fluid flow within the ECM may stimulate biosynthesis via several mechanisms, one possible mechanism may be related to increased convective transport of nutrients and growth factors\textsuperscript{43}. Bernich et al.\textsuperscript{44} observed that pressure-induced fluid flow through cartilage disks \textit{in vitro} could greatly enhance the transport of glucose and other small nutrients through the tissue. Maroudas and coworkers measured the effects of static\textsuperscript{67} and dynamic\textsuperscript{65} compression on the partitioning and absorption of large and small molecules into cartilage, including radio-labeled BSA, IGF-I, urea, and sodium. They concluded that static compression affected the transport of large solutes more than that of small solutes, and that dynamic compression enhanced the desorption of large solutes much more than small solutes. If convective transport is an operative metabolic stimulant during dynamic compression of cartilage, it might act by (1) directly stimulating chondrocytes (for example, fluid shear at the cell surface\textsuperscript{68}), or (2) altering the pericellular concentrations of macromolecular cytokines, growth factors, degradative enzymes, endogenous enzyme inhibitors, newly synthesized matrix macromolecules, or other nutrients.

Recently, Garcia \textit{et al.} developed an approach to quantify the individual contributions of diffusion, convection, and electrical migration to the transport within cartilage of neutral and charged proteins, and lower MW solutes\textsuperscript{69}. This approach allows direct measurement within each experiment of solute diffusion, convection (by application of an electric current to induce electro-osmotic fluid flow within the tissue (Fig. 7.25(a))), and electrical migration of charged solutes (which would occur in the presence of streaming potentials). Their results showed that convective enhancement of transport was particularly important for larger solutes. However, the effects of fluid convection became significant for solutes as small as 300–600 Da\textsuperscript{69}. They further observed\textsuperscript{70} that protein flux within cartilage could be greatly enhanced by fluid velocities relevant to physiologic mechanical loading. For example, transport of \textsuperscript{125I}-IGF-1 and \textsuperscript{125I}-rTIMP-1 were enhanced by approximately 20- and 60-fold, respectively, above diffusion alone, by fluid velocities of about 1–2 µm per second. Fig. 7.25(b) shows the initial diffusive flux of IGF-1 across cartilage disks mounted in a transport chamber (from t = 590 to t = 745 minutes), followed by a 20-fold increase in flux at t = 745 minutes produced by application of electro-osmotic fluid flow within and across the cartilage disks\textsuperscript{70}.

**Intracellular pathways: differential effects of compression on specific ECM macromolecules**

The ability of the matrix to adapt to biomechanical demands is likely related to observations that the resident chondrocytes are capable of responding to specific physical stimuli within the tissue. Most studies of the effects of compression on cartilage biosynthesis \textit{in vitro} have focused on total PG and protein synthesis using radiolabel precursors. However, some recent studies have begun to address the differential effects of compression on synthesis of specific matrix molecules, including biglycan and decorin\textsuperscript{71,72}, fibromodulin\textsuperscript{71}, fibronectin\textsuperscript{40}, hyaluronan and link protein\textsuperscript{53}, and of of \textsuperscript{35}S-aggrecan and \textsuperscript{3}H-collagen metabolism.

The three molecular components of the proteoglycan aggregate, for example, involve very different intracellular biosynthetic pathways. Link protein, a typical glycoprotein, undergoes a set of well-defined post-translational steps of N-linked oligosaccharide addition and trimming prior to its secretion from the cell. On the other hand, the post-translational processing of aggrecan core protein is spatially and temporally much more elaborate, requiring sequential addition of N-linked oligosaccharides, chondroitin sulfate (CS), O-linked oligosaccharides, and keratan sulfate (KS)\textsuperscript{73}. (Synthesis of CS is initiated in the late endoplasmic reticulum and continued in the proximal regions of the Golgi complex\textsuperscript{73}, and KS is added to preformed O-linked oligosaccharides in the trans-Golgi in close spatial and temporal association\textsuperscript{42,72}.) In marked contrast to both aggrecan and link protein, hyaluronan does not involve a protein precursor. Rather, hyaluronan is synthesized at the plasma membrane by hyaluronate synthase and secreted directly into the extracellular matrix\textsuperscript{76}.
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Cellular mechanisms: biophysical, morphological, and biosynthetic correlates

Cell and nucleus deformation at equilibrium follows imposed tissue deformation

The effects of tissue compression on the deformation of the matrix, chondrocyte, and intracellular components have been studied to better understand the possible role of cell shape / deformation on chondrocyte signal transduction. Using Nomarski imaging, confocal microscopy, and stereology of explant specimens fixed after static and dynamic compression, investigators have found that compression applied to the surfaces of cartilage specimens causes a corresponding compression of the pericellular, as well as territorial and interterritorial, matrix, near and around the cells. In adult cartilage, columns of chondrocyte-containing chondrons appear compacted at all depths with accompanying loss of pericellular matrix volume and water content. Recent studies have shown that distinct changes in cell and nucleus shape are produced by compression imposed at tissue surfaces. In general, compression caused flattening of the cells in the direction of loading and a decrease in cell volume, and could also cause changes in cell surface area, nucleus volume and height, and nucleus surface area. These changes vary with depth, the degree of anisotropy of the collagen network, and the age of the tissue.

Buschmann et al. have directly examined whether such changes in cell and nucleus morphology are related to the changes in aggrecan biosynthesis caused by static compression. Cartilage explant disks were radiolabeled during compression, fixed in the compressed state (Fig. 7.27), and subsequently processed for quantitative autoradiography and light microscopy-based stereological estimations. Analysis of light microscope images was used to measure sulfate autoradiography grain density (associated with incorporation of newly synthesized proteoglycans into the matrix). They found a general inhibition of total aggrecan synthesis with increasing static compression levels, and a compression-induced spatial gradient in aggrecan synthesis which was not present in fresswelling disks. This spatial inhomogeneity appeared to be directly related to mechanical boundary conditions and the manner in which the load was applied and,
therefore, may represent a spatially specific functional adaptation to mechanical loading. Stereological measurements at the center of disks showed a reduction in cell and nucleus volume, surface area, and radii in the vertical direction of compression. Horizontal radii were unchanged, however, consistent with the observation of minimal radial expansion of the explant disks in (unconfined) compression. Thus, vertical and horizontal cell dimensions in compressed tissue followed deformations imposed at the tissue surfaces. These correlated changes in biosynthesis and stereological parameters suggest that alterations in cell and nuclear structure may be an important mode by which chondrocytes can detect and respond to changes in the mechanical environment.

As described above, proteoglycan synthesis is generally decreased by static compression, while dynamic compression can stimulate synthesis. Quantitative autoradiography has enabled investigators to characterize the spatial profiles of aggregcan biosynthesis within the tissue, caused by dynamic\textsuperscript{37}, as well as static\textsuperscript{31} compression. Quinn\textsuperscript{42} recently extended these methods to quantify the spatial distributions of incorporated radiolabel around individual cells, within 3 mm diameter by 1 mm thick explant disks, that had been subjected to static or dynamic compression (Fig. 7.28). \textsuperscript{35}S-sulfate autoradiography grain density was measured (with about 1 μm resolution) in the cell-associated and further removed matrix, as a function of distance from the cell membrane and angular orientation around centrally sectioned cells. Systematic random sampling\textsuperscript{43} over the entire cross-section of explant disks allowed for the identification of trends associated with cell populations from particular explant locations.

Analysis of compressed cartilage disks revealed that the spatial distribution of newly synthesized proteoglycans incorporated around individual chondrocytes, was strongly influenced by the orientation of mechanical compression which was applied to the whole disk in the thickness direction. Static compression decreased the content of newly synthesized proteoglycans found in both the cell-associated and further removed matrices of cells in the central region of explant disks (Fig. 7.28(a)(iii)), compared to that in free-swelling controls (FSW) (Fig. 7.28(a)(i)). However, cells near the radial edge (outer 25 per cent of the 3 mm diameter cross-section) of statically compressed explants (Fig. 7.28(a)(iv)), where anisotropic matrix deformations would be maximum, exhibited a pronounced directional character with respect to inhibition of cell-associated proteoglycan incorporation, which was not evident near the center of compressed explants (Fig. 7.28(a)(iii)), nor in free-swelling samples (Fig. 7.28(a)(i)). At the radial edge, the deposition of newly synthesized proteoglycans was inhibited significantly at the ‘north’ and ‘south’ poles of the cells (that is, in the axial direction of compression) (Fig. 7.28(a)(iv)) compared to free-swelling controls (Fig. 7.28(a)(ii)), but not significantly altered around the ‘equators’ of the flattened cells (Fig. 7.28(a)(iv)). Figure 7.28 (b) shows the effect of dynamic compression at 0.1 Hz on the distribution of newly synthesized proteoglycans around cells. Previous studies at the tissue level (Fig. 7.24(c)) had shown that proteoglycan synthesis was stimulated at this frequency predominantly in the outer radial ring of 3 mm diameter explants. At the cell level, Quinn found that this stimulation of proteoglycan synthesis was manifested by a marked increase in the content of newly synthesized proteoglycans in the cell-associated matrix of these cells (Fig. 7.28(b)(iv)). The increased content at the ‘equators’ was not that different from the ‘poles’. In contrast, cells in the central region of dynamically compressed disks (Fig. 7.28(b)(iii)) showed little change in content of newly synthesized proteoglycans, consistent with the lack of stimulation in the central region at the tissue level (Fig. 7.24(c)). These microscale data highlight the complexity of the chondrocyte’s micromechanical and biochemical environment, and suggest the importance of fluid flows in the pericellular matrix during dynamic compression (Fig. 7.25), as well as mechanical deformations, as potential mediators of metabolic activity.

Comparison of changes in aggregcan synthesis at the center of cartilage disks, to changes in cell and nucleus structure at the same location, showed that aggregcan synthesis was reduced simultaneously with reductions in cell and nucleus volumes, surface areas, and vertical radii.\textsuperscript{41} Based on these data alone, however, it is difficult to establish a direct cause and effect relationship between biosynthesis and any one structural parameter. Nevertheless, the distinct correlation between changes in aggregcan synthesis, and general cell and nucleus structure, serves to highlight a number of potentially important control points and molecular mechanisms which may link synthesis to structure of the cell or nucleus\textsuperscript{41}. The chondrocyte / extracellular-matrix interface bears integrin\textsuperscript{44,45} and hyaluronan receptors\textsuperscript{46}. The cytoplasmic domain of integrin receptors is linked directly to the cell cytoskeleton and possesses a tyrosine kinase activity\textsuperscript{47}. Mechanical per-
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In OA, cartilage matrix composition is altered substantially, considerably weakening the tissue to the extent that mechanical wear from joint motion can result in erosion of cartilage down to the bone surface. Cartilage matrix molecules are susceptible to degradation by several classes of proteases. The role of enzymatic degradation in OA is under intense investigation, but is not completely understood. It has been suggested that families of enzymes, including metzincins, serine proteases, and a novel 'aggreganase' contribute to cartilage degradation in OA. It is known that a portion of the aggregan G1 domain of aggregan that accumulates in human cartilage is associated with matrix metalloproteinase activity. However, the aggregan fragments that are present in the synovial fluid of early and late post-traumatic arthritis and inflammatory arthritis contain the cleavage site consistent with 'aggreganase' activity. The role of mechanical agents in the degradation of cartilage in vivo has recently been reviewed. It is clear that acute mechanical overload can cause severe cartilage damage. Recent studies in vitro have simulated the effects of controlled impact loads on cartilage explants to assess matrix fissuring, chondrocyte viability, and damage to the collagen network. Other than such acute destruction, the mechanisms by which mechanical forces in the joint may contribute to specific catabolic pathways for matrix degradation remain to be elucidated. It is possible that mechanical compression could alter enzymatic pathways and, thereby, the forms of the catabolic fragments of aggregan, link protein, hyaluronan, and collagen. In this regard, it is important to distinguish between the direct effects of mechanical load in disrupting cartilage matrix and mechanical stimulation of cell-mediated catabolic pathways. For example, high amplitude cyclic compression of cartilage explants has been found to...
produce a dose-dependent sustained release of aggrecan and collagen fragments, resulting in tissue swelling, as well as increased release of aggrecan G1 domain fragments. Analysis of the specific cleavage sites associated with such released macromolecular fragments can give insight as to whether compression has induced 'mechanically novel' catabolic fragments, or has simply accelerated the loss of fragments found during normal turnover of matrix. Further research in these areas will be essential for a more complete understanding of the chondrocyte response to mechanical loads.

Acknowledgments

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References

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Fig. 7.23

Branicki
(a) 35S incorporation: oscillatory/static

\begin{align*}
\text{Frequency (Hz)} \quad &\leq 0.001 \quad 0.01 \quad \geq 0.1 \\
\text{incorporation ratio} &\quad 1 \quad 1 \quad 1
\end{align*}

(b) Normalized pressure and fluid velocity and potential gradient

(c) Proteoglycan synthesis (dynamic/static)

\begin{align*}
r = 0 \quad 0.5 \quad 1.0 \quad 1.5 \text{ mm} \\
\text{synthesis ratio} &\quad 1 \quad 1 \quad 1 \quad 1.25
\end{align*}

\text{p < 0.001}

NS
Direction of applied compression

Autoradiograph: disk cross-section n

(a) Static compression grain density (µm^2)

Center of explant

Radial edge

i FSW
n = 35

ii FSW
n = 40

iii 1 mm
n = 29

iv 1 mm
n = 29

Distance from cell membrane (µm)

(b) Dynamic compression grain density (µm^2)

Center of explant

Radial edge

i 10 Hz
n = 34

ii 0 Hz
n = 35

iii 0.1 Hz
n = 37

iv 0.1 Hz
n = 29

Bravdey fig 7.28