Full Length Article

Haversian remodeling corresponds to load frequency but not strain magnitude in the macaque (*Macaca fascicularis*) skeleton

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A B S T R A C T

One way bone adapts to its mechanical environment is by Haversian remodeling, a repair process in which existing bone is resorbed and replaced by new bone. Haversian remodeling forms interconnected, cylindrical structures called secondary osteons. The amount of remodeling that occurs is related to the nature of mechanical loading and accrual of microdamage, but it is uncertain whether habitual loads of high magnitude versus high frequency result in more remodeling. The answer to this question is important if remodeling is to be a tool for inferring loading environments, and thus behavior, in past populations. Here, secondary osteon population density (OPD), osteon cross-sectional area (On.Ar), and percent Haversian bone (%HAV) were compared among mid-diaphysis femora, tibia, fibulae, and mid-level ribs of five adult crab-eating macaques (*Macaca fascicularis*). Ribs experience relatively low strains but have a high daily loading frequency (~33 times per minute). Limb bones are loaded for fewer cycles per day, but the femur and tibia have high load magnitudes due to gravitational forces. Strain magnitudes in the fibula are a fraction of those in the femur and tibia. Analyses of variance demonstrated significant differences in OPD ($P = 0.010$) and On.Ar ($P < 0.001$) among the bones. Pairwise t-tests revealed greater OPD but lower On.Ar in the rib than all other bones. The high rib OPD suggests that Haversian remodeling is more responsive to load frequency than strain magnitude. The fact that osteons are smaller in ribs than any other bone may be an effect of remodeling in comparatively narrow cortices.

1. Introduction

Bone is a dynamic tissue that can respond to mechanical loads it encounters throughout an individual's life. This plasticity allows the skeleton to adapt and maintain its structural integrity under a variety of loading conditions [1,2]. Investigating the functional connections between behavior, loading regimes, and patterns of bone modeling and remodeling can inform behavioral interpretations in past populations (i.e., the fossil and archaeological records). Bone remodeling is known to be related to mechanical loading; however, the extent to which various loading parameters (i.e., load magnitude, strain frequency, rate, and mode) influence remodeling activity remains uncertain. The question explored here is whether increased remodeling activity is associated with high strain magnitude or high loading frequency.

Haversian bone remodeling is the coordinated activity of osteoblasts and osteoclasts to resorb and replace existing cortical bone. This process aids in mineral homeostasis by releasing calcium reserves [3] but also

serves biomechanical functions, allowing bone to adapt at the microstructural level. One way remodeling does this is by repairing bone – targeting areas with microdamage caused by mechanical deformation [4–8]. Remodeling results in the formation of interconnected, cylindrical structures called secondary osteons. Secondary osteons are concentric lamellae surrounding a central Haversian canal that houses a neurovascular bundle, and are encompassed by a visible cement line, which is a remnant of the reversal from resorption to formation. The presence of secondary osteons can help prevent further damage in two ways. First, since the newly laid bone is less mineralized than older bone, and lower mineralization means greater compliance [8], the presence of secondary osteons increases bone toughness [9–14]. Additionally, osteon cement lines can serve as barriers to crack propagation by dissipating the energy from a growing crack around the cement line, rather than permitting it to continue through the bone matrix [8,15–17].

The majority of bone remodeling throughout the skeleton has been

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hypothesized to be associated with bone microdamage [18]. Both high strains [19–22] and cyclical loading [23] can cause microdamage. Thus, significant remodeling is expected to occur in regions of the skeleton that experience relatively severe loading conditions. It has also been hypothesized [24,25] that osteon size is inversely related to strain magnitude, such that regions of high strain will have small osteons. The question is whether highly remodeled bone has been the subject of elevated strains or highly repetitive loading cycles. Lad et al. [26] found very little remodeling in the mandibular symphysis of Old World monkeys, a region of the facial skeleton that experiences relatively high strains. Additionally, they found that colobines, which have relatively high mastication frequencies, have more remodeling in the mandibular corpus than cercopithecines, including the sooty mangabey (*Cercocebus atys*), which engages in powerful (high force magnitude) bites while consuming very hard seeds. The authors hypothesized that remodeling activity is more closely tied to the frequency of loading than to strain magnitude. Lad et al. [27] presented conflicting evidence about the relationships of load magnitude and load frequency to osteon size and density in the femur and humerus of cercopithecid primates. Femora of leaping species were found to have more secondary bone than in species that leap less, suggesting that high propulsive forces engender more remodeling. However, in all species there was more secondary bone in the humerus than the femur, despite the hindlimbs routinely experiencing greater peak loads than the forelimbs [28–31]. That finding suggests that the activation of bone remodeling might depend on factors other than load magnitude.

Hypotheses about the relationship of load magnitude and frequency to remodeling are tested here by comparing the amount of secondary bone in the cortices of four bones in the macaque skeleton: femur, tibia, fibula, and rib. Crab-eating macaques are highly arboreal primates with a locomotor regime consisting primarily of quadrupedalism and vertical climbing [32,33]. The femur and tibia are weight-bearing bones and endure greater gravitational loads and impact forces than either the fibula or rib. The macaque tibia experiences maximum peak principal strains of $1272 \pm 424\mu\varepsilon$ during a gallop [34]. In vivo strain data are not available for the macaque femur but it presumably experiences high strains as well since peak principal strains in radius, tibia, and femur of other mammalian quadrupeds range from -3200 to $-2100\mu\varepsilon$ during running, trotting, and galloping [35].

The fibula bears a fraction of the axial loads applied to the femur and tibia, with estimates of maximum proportion of forces transmitted through the limb ranging from 13%–17% [36–39]; thus, strains in the fibula are presumably lower, even considering its relatively small caliber. However, it is loaded with the same frequency as the tibia and femur in terms of cycles per day; i.e., with each stride.

Ribs are non-weight bearing bones that do not experience impact loads. While there are no in vivo strain gage data for ribs, ex vivo strain amplitudes in human ribs in simulated breathing are less than $500\mu\varepsilon$ [40], well less than half the peak strains observed in limb bones. Ribs almost certainly experience strain from muscle contractions (e.g., serratus anterior, external abdominal oblique) in the thorax, which may exceed that generated during respiration, but the most frequent source of deformation of the ribs is certainly breathing. During inhalation and exhalation the entire rib cage expands and contracts, respectively. Skedros et al. [41] found higher number of microcracks in mule deer ribs than in bone of the forelimb, one explanation for which was fatigue damage from thoracic movement during breathing. Despite the low strain magnitudes, the ribs have high loading frequency as macaques breathe at a rate of 33 cycles per minute [42], which presumably equates to more loading cycles per day than locomotor behaviors generate in any of the limb elements.

The four bones can thus be sorted in terms of strain magnitude, with the femur and tibia having high strains and the fibula and rib having low strains, or in terms of load frequency, with the rib having high load frequency and the other bones having lower load frequency. The frequency and magnitude of mechanical deformation are both expected to

contribute to microdamage formation, and bone remodeling is potentially linked to the interaction of these variables, which can be summarized as strain rate (i.e., deformation over time, or the change in strain that occurs in a given time) [43–45]. However, our goal presently is to illuminate the respective contributions of load magnitude and frequency to remodeling activity.

Three hypotheses are tested by comparing the relative amount of secondary bone in each of these four bones [1]. The null hypothesis is that neither strain magnitude nor strain frequency effects Haversian remodeling in the macaque skeleton, and instead there is a systemic rate of remodeling throughout the skeleton. If this is true, then we expect no differences in the amount of secondary bone among the femur, tibia, fibula, and rib [2]. An alternative hypothesis is that Haversian remodeling depends predominantly on strain magnitude, rather than load frequency. Under this hypothesis, the femur and tibia are expected to have more secondary bone and larger osteons than the fibula or rib because strains are relatively low in the latter two elements [3]. A second alternative hypothesis is that remodeling activity largely depends on load frequency, rather than strain magnitude. If this holds true, then the rib should have more secondary bone than other skeletal elements because of its high load frequency. The fibula, tibia, and femur should have fairly equal amounts of secondary bone.

2. Materials and methods

2.1. Sectioning methods

The sample comprises the left femur, tibia, fibula, and one mid-level rib from each of five female macaque (*Macaca fascicularis*) skeletons belonging to the primate skeletal collection in the Department of Anthropology at The Ohio State University. Individuals are adults based on epiphyseal fusion but exact age-at-death is unknown. Thick sections (ca. 1 mm) were cut from each bone at 50% the length of the whole bone for the femur, tibia, and fibula, and from the middle third of each rib. Each thick section was embedded in Buehler Epoxicure resin for protection and ease of manipulation. The embedded sections were cut to a thickness of 100 μm using a Buehler Isomet low speed saw (Illinois Tool Works, Lake Bluff, IL) and diamond wafering blade. The thin sections were then polished on a Buehler MetaServ 250 grinder-polisher to remove debris and markings from the saw blade. The sections were stained with a toluidine blue O solution to increase visibility of secondary osteon cement lines, and then dried under a 340 g weight to prevent warping. Thin sections were mounted to microscopy slides with cytoseal 60 and xylene-dipped cover slips were placed on top.

2.2. Data collection

The thin sections were photographed under compound light microscopy at $100\times$ magnification. A series of overlapping photographs was taken of the entire area of each thin section so that the bone cortex was completely captured. The photographs for each section were compiled into one composite image using PTGui Photo Stitching Software to ensure that no area was measured more than once. A 1 mm scale bar was also photographed and used to set the scale for size measurements.

The composite images were analyzed in ImageJ visualization software [46]. The entire cortex was traced to measure total area, and each secondary osteon and fragmentary osteon was counted and traced to measure the area within the cement line. Secondary osteons and osteon fragments were distinguished from primary osteons by the presence of a cement line. Three variables were calculated from these measurements: osteon population density (OPD), percent Haversian bone (%HAV), and osteon cross-sectional area (On.Ar). OPD is the total number of secondary osteons and osteon fragments divided by total cortex area. % HAV is the total area of secondary bone (area of all bone within the cement lines of secondary osteons and fragmentary osteons) divided by

Table 1
Osteon population density (OPD) and percent Haversian bone (%HAV) in midshaft thin sections of macaque (*Macaca fascicularis*) bones.

Specimen	OPD	%HAV
Femur		
1	5.59	11.66
2	3.86	8.71
3	8.29	17.26
4	3.70	7.62
5	5.56	11.89
Tibia		
1	7.33	12.31
2	4.64	10.38
3	13.49	31.88
4	6.00	13.50
5	9.07	23.16
Fibula		
1	9.59	18.28
2	6.42	14.89
3	25.49	51.06
4	3.87	6.55
5	18.64	43.21
Rib		
1	15.78	28.78
2	15.12	25.33
3	20.09	26.05
4	16.25	23.10
5	19.69	36.79

Table 2
Osteon cross-sectional area (On.Ar) in mm^2 in midshaft thin sections of macaque (*Macaca fascicularis*) bones.

Specimen	Mean On.Ar	SD	Range
Femur			
1	0.021	0.0117	0.005–0.058
2	0.023	0.0128	0.004–0.073
3	0.022	0.0125	0.004–0.063
4	0.021	0.0118	0.005–0.066
5	0.022	0.0133	0.004–0.129
Tibia			
1	0.018	0.0092	0.004–0.043
2	0.023	0.015	0.004–0.082
3	0.025	0.0119	0.005–0.078
4	0.023	0.0153	0.005–0.119
5	0.027	0.0169	0.004–0.122
Fibula			
1	0.02	0.0118	0.004–0.072
2	0.024	0.0154	0.005–0.066
3	0.023	0.0124	0.007–0.074
4	0.019	0.0118	0.005–0.051
5	0.025	0.0148	0.004–0.075
Rib			
1	0.019	0.0115	0.006–0.059
2	0.018	0.007	0.006–0.035
3	0.014	0.0068	0.004–0.036
4	0.014	0.0074	0.005–0.032
5	0.019	0.0096	0.004–0.058

the total bone area, multiplied by 100 and expressed as a percentage. On.Ar is the area of a complete secondary osteon reported in mm^2.

2.3. Statistical analysis

Shapiro-Wilk tests for normality [47] were performed on all data and those that failed to meet assumptions of normality were z-rank transformed using R package GenABEL. One-way analyses of variance (ANOVAs) were performed with OPD, %HAV, and On.Ar as dependent variables and bone as the independent variable to test for differences in means among femora, tibiae, fibulae, and rib (Null Hypothesis). In the event of significant ANOVA results, post-hoc two-tailed pairwise *t*-tests were performed to determine which (if any) bones had greater OPD relative to others (Alternative Hypotheses).

3. Results

OPD and %HAV data are presented in Table 1 and On.Ar data are in Table 2. The femur has the lowest mean OPD (5.40), followed by the tibia (8.11), fibula (12.80), and then rib (17.39) (Fig. 1). Mean %HAV is, from least to greatest, 11.43% in the femur, 18.25% in the tibia, 26.80% in the fibula, and 28.01% in the rib. Mean On.Ar is $0.0171mm^2$ in the rib, $0.0215mm^2$ in the femur, $0.0235mm^2$ in the fibula, and $0.0236mm^2$ in the tibia (Fig. 2).

OPD and On.Ar failed Shapiro-Wilk tests (Table 3) and were z-rank transformed to normalize distributions. ANOVAs returned significant results for OPD ($P = 0.010$) and On.Ar ($P < 0.001$), but not for %HAV ($P = 0.101$), indicating that the density and size of osteons differs among bones but the overall amount of secondary bone does not. Pairwise *t*-tests revealed that for OPD (Table 4) the femur is significantly different from the rib and fibula, and the tibia and rib are significantly different from each other. Fibula-rib, tibia-fibula, and tibia-femur comparisons were not significant. Pairwise *t*-test results for On.Ar (Table 5) showed that the femur and rib are both significantly different from all other bones. The lowest On.Ar is the same in all bones: $0.004mm^2$. However, the largest On.Ar is comparable in the femur and tibia ($0.129mm^2$ and $0.122mm^2$, respectively) but lower in the fibula ($0.075mm^2$), and much lower in the rib ($0.059mm^2$). The lack
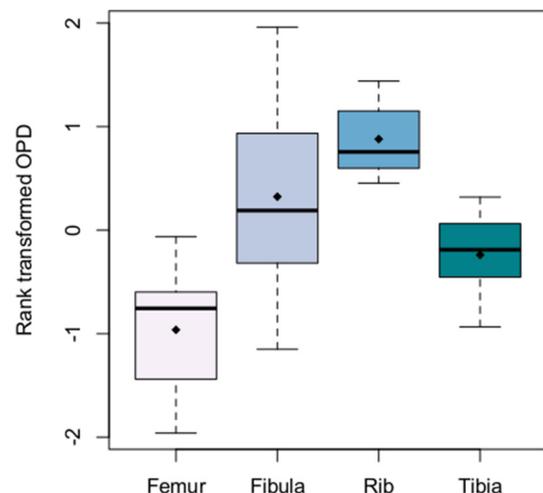

Fig. 1. Boxplot of osteon population density (OPD) in macaque femora, tibiae, ribs, and fibulae. OPD is significantly different among the bones ($P = 0.010$). OPD data are z-rank transformed. The bottom and top of the boxes indicate the interquartile range, the horizontal bar is the median, the diamond is the mean, and the whiskers represent minimum and maximum data points.

of significant differences in %HAV is a result of the small sizes of the densely-packed rib osteons and the larger but more sparse osteons in the femur and tibia.

4. Discussion

The Null Hypothesis, that strain magnitude and frequency do not affect bone remodeling, can be rejected because there were differences in the amount of secondary bone among the femur, tibia, rib, and fibula. This result suggests that the unique load case of each bone may influence bone remodeling differently, and that the rate of remodeling is not uniform throughout the skeleton. Alternative Hypothesis 1, which stated that remodeling is primarily dependent on strain magnitude, can also be rejected. The two weight-bearing bones, femur and tibia, which
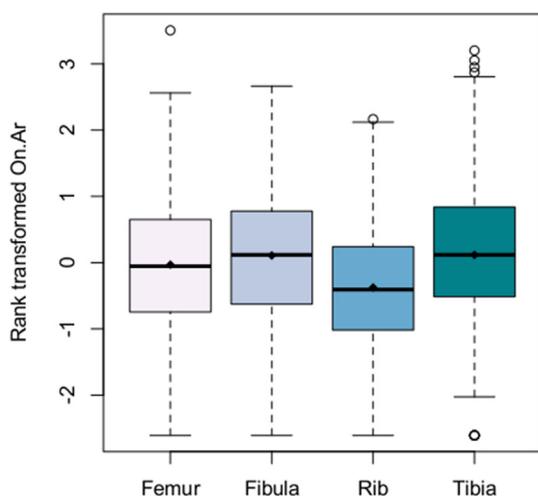
Fig. 2. Boxplot of osteon cross-sectional area (On.Ar) in macaque femora, tibiae, ribs, and fibulae. On.Ar is significantly different among the bones ($P < 0.001$). On.Ar data are z-rank transformed. The bottom and top of the boxes indicate the interquartile range, the horizontal bar is the median, the diamond is the mean, and the whiskers represent minimum and maximum data points.

Table 3
Shapiro-Wilk normality test results for all data.

Variable	Result	P-value
OPD	Failed	0.033
%HAV	Passed	0.068
On.Ar	Failed	< 0.001

Table 4
Pairwise *t*-test *P*-values for osteon population density (OPD) in the femur, tibia, fibula, and rib. Alpha is 0.05. OPD is significantly lower in the femur than in the fibula and rib, but not significantly different from the tibia. The tibia is significantly lower than the rib, but not different from the fibula. The fibula and rib are not significantly different.

	Femur	Fibula	Rib
Fibula	0.018*	–	–
Rib	0.002**	0.269	–
Tibia	0.156	0.265	0.035*

Asterisks indicate the level of significance of the p-values (*less than 0.05, **less than 0.01, ***less than 0.001).

Table 5
Pairwise *t*-test *P*-values for osteon cross-sectional area (On.Ar) in the femur, tibia, fibula, and rib. Alpha is 0.05. On.Ar is significantly lower in the rib than in all other bones. The tibia and fibula do not differ from each other but both have significantly greater On.Ar than the femur.

	Femur	Fibula	Rib
Fibula	0.029*	–	–
Rib	< 0.001***	< 0.001***	–
Tibia	0.003**	0.880	< 0.001***

Asterisks indicate the level of significance of the p-values (*less than 0.05, **less than 0.01, ***less than 0.001).

likely have higher peak strains than either fibula or rib, do not have high osteon densities. Rather, the opposite was found: the rib, which has the greatest loading frequency, but does not directly bear body weight or gravitational forces, has significantly more secondary bone than either the tibia or the femur. The relatively high OPD in the rib provides strong support for Hypothesis 2: remodeling activity depends predominantly on strain frequency, rather than strain magnitude.

Interestingly, the fibula did not differ from the tibia and had greater OPD than the femur despite having presumably equal loading frequency, yet a fraction of the axial loads experienced by the tibia and femur. This finding could suggest that high strain amplitudes are not necessary for high degrees of remodeling to occur, which is consistent with data from cercopithecine monkey species [26] which indicated very little remodeling in high strain regions of the mandible. However, gravitational forces are not the only stimulus for bone adaptation; muscular activity can also cause significant bone deformation [48]. One explanation for the relatively high incidence of secondary bone in the fibula could be that strains generated by soft tissue are higher in the fibula than other limb bones. We are not aware of any data that support this hypothesis. Another explanation could be that while axial loads are *absolutely* lower in the fibula than the tibia or femur, the fibula is far less robust than the other two bones. Thus, a relatively small load could theoretically result in equivalent or greater strain in the fibula than in the femur or tibia. Lieberman et al. [49] found greater osteon densities in distal limb segments in cursorial animals (sheep) in which there is a tapering effect (i.e., decreased cortical areas in more distal limb elements). In vivo strain data would elucidate whether a similar effect is at play here; i.e., the lower robusticity of the fibula results in higher strain than expected given the applied loads. If this were true, the hypothesis that strain magnitude is less important would need to be revisited.

There was no strong evidence for a relationship between osteon size and loading frequency or magnitude. The relatively small osteons found in ribs could suggest that high load frequency results in smaller osteons, but it is unclear why increased loading would result in smaller, rather than larger, resorption spaces. It has been predicted that smaller osteons would be beneficial in areas of heavy microdamage accumulation because small, densely packed osteons provide more cement lines which act as barriers to crack propagation, effectively toughening bone [16,17]. Larger osteons are also more prone to "pull out" [50], a phenomenon in which the osteon becomes debonded from the surrounding matrix and bridges a crack [51]. Osteon pull out tends to occur more frequently under tension than compression [52,53], which may be a source of variation in osteon size. In fact, variations in osteon size have been primarily linked to variations in strain mode, with osteons tending to be smaller in regions of compression and larger in regions of tension [24,54,55].

There is no reason to suspect that ribs experience predominantly compressive strain whereas the limbs experience tensile strain, so variations in strain mode are unlikely to explain the finding of smaller osteons in the ribs. Bending in vivo likely characterizes all these elements to a large degree. An alternative explanation is that cortical width constrains osteon size [56]. Resorption space dimensions are limited by cortical thickness, because resorption space diameters approaching cortical thickness jeopardize the structural integrity of the bone [57]. For example, a resorption space with an area of $0.10 mm^2$ is more likely to cause deleterious effects in a cortex that is 0.50 mm wide (rib) than in a cortex that is 1.5 mm wide (femur). The fact that osteons do not get nearly as large in ribs as they do in other bones may indicate a biomechanical constraint on osteon size.

These results provide evidence for a link between loading frequency and bone remodeling, and suggest that the bony response to loading frequency versus strain magnitude should be further explored. However, there are two limitations to this study that should be improved upon for future work on this topic. First, due to the destructive nature of histological analyses and the opportunistic nature in which wild samples are collected, the sample size is small. Larger samples would allow for more statistical power in future testing of the hypotheses addressed here. Second, in vivo strain data for all of the bones examined here are lacking for the species studied. While relative strain magnitudes and frequencies have been inferred from what data are available (from other species or ex vivo studies), it should be noted that different activity can change the relative peak strain in different skeletal

elements. Consequently, strain can be higher than expected based on inference. For example, Biewener et al. [58] demonstrated that peak stress in the horse radius is substantially greater (-41 MN m^{-2}) than in the metacarpus (-25 MN m^{-2}) during steady state locomotion, but peak stress in the metacarpus during acceleration is significantly greater (-40 NM m^{-2}). Those authors concluded that both bones have similar safety factors to failure when the highest strains are encountered. This specificity of information is lacking in this study and future efforts to understand the relative contributions of loading frequency and strain magnitude to bone remodeling activity would benefit from in vivo data.

Finally, loading frequency and strain magnitude are not the only loading parameters that can affect bone remodeling activity and the distribution of secondary osteons. Cortical bone is known to have different mechanical properties, fracture properties, and microdamage effects under different strain modes, especially in tension versus compression [23,53,59–64]. With regards to microdamage, bone in tension has a lower yield strength compared to bone under compression [65,66] and fatigue life is shorter than that of bone under compression at the same load magnitude [67,68]. Microdamage tends to accumulate more rapidly in regions of tension but microcracks that form under compression tend to be longer, are less often obstructed by osteon cement lines, and cross lamellae, whereas cracks in tension are more likely to debond lamellae from each other [23,62]. Different rates of microdamage accumulation and microcrack growth under distinct strain modes may result in correspondingly different rates of bone remodeling. There is evidence that the spatial distribution of secondary osteons in limb bones corresponds to regional strain modes, with greater secondary osteon densities found in regions of compression than tension [54,55,69]. An additional complication is that bones with "high load complexity" [70] have less predictable loading, resulting in multidirectional bending or torsion. The present study is limited in that these other strain-related parameters are unknown. A future line of research will assess the spatial distribution of secondary osteons in the macaque skeleton to determine whether strain mode, in addition to loading frequency, is responsible for remodeling patterns in bones for which the in vivo load case is known.

In sum, we present evidence that the incidence of bone remodeling in the crab-eating macaque skeleton may be more closely tied to the frequency of mechanical loading, rather than the magnitude of strain, as evidence by the high density of secondary osteons in the rib. Further, high osteon density in the fibula compared to the femur suggests that high strains are not requisite for remodeling to occur. Patterns of bone remodeling may be useful for investigating questions about the frequency of loading and associated behaviors, but additional work is required to determine exactly how various strain parameters dictate remodeling patterns before it can be used as a tool for behavioral inference in past populations.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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