

Identification of interleukin 2, 6, and 8 levels around miniscrews during orthodontic tooth movement

Nihal Hamamcı*, Filiz Acun Kaya**, Ersin Uysal*** and Beran Yokuş****

Departments of *Orthodontics, **Periodontology, ***Computerised Programming and ****Biochemistry, Dicle University, Diyarbakır, Turkey

Correspondence to: Dr Nihal Hamamcı, Department of Orthodontics, School of Dentistry, Dicle University, 21280 Diyarbakır, Turkey. E-mail: nhamamci@dicle.edu.tr

SUMMARY The aim of this study was to identify the levels of interleukin (IL)-2, IL-6, and IL-8 around miniscrews used for anchorage during canine distalization.

Sixteen patients (eight males and eight females; mean age, 16.6 ± 2.4 years) who were treated with bilateral upper first premolar extractions were included in the study. Thirty-two maxillary miniscrew implants were placed bilaterally in the alveolar bone between the maxillary second premolars and first molars as anchorage units for maxillary canine distalization. Three groups were constructed. The treatment, miniscrew, and control groups consisted of upper canines, miniscrew implants, and upper second premolars, respectively. Peri-miniscrew implant crevicular fluid and gingival crevicular fluid (GCF) were obtained at baseline (T1) and at 1 (T2), 24 (T3), and 48 (T4) hours, 7 (T5) and 21 (T6) days, and 3 months (T7) after force application. Paired sample *t*-tests were used to determine within-group changes and Dunnett's *t* and Tukey's honestly significant difference tests for between-group multiple comparisons.

During the 3 month period, IL-2 levels significantly increased ($P < 0.01$) but only in the treatment group after 24 hours. IL-6 levels were unchanged at all times points in the three groups. IL-8 levels increased significantly at 1 ($P < 0.05$), 24 ($P < 0.01$), and 48 ($P < 0.01$) hours in the treatment group and at 24 ($P < 0.05$) and 48 ($P < 0.01$) hours in the miniscrew group. It appears that miniscrews can be used for anchorage in orthodontics when correct physiological forces are applied.

Introduction

Cytokines are involved in initiating, amplifying, perpetuating, and resolving inflammatory responses in periodontal and peri-implant tissues (Panagakos *et al.*, 1996; Graves and Cochran, 2003). They are key mediators of tissue damage and play an important role in tooth movement. Cytokines are classified as pro- and anti-inflammatory. Proinflammatory cytokines include tumour necrosis factor, interleukin (IL)-1, IL-2, IL-6, and IL-8. These are 'alarm' cytokines that induce vascular dilation through increased permeability and enhanced inflammatory response (Stoycheva and Murdjeva, 2005).

IL-2 is a proinflammatory cytokine produced by T-helper 1 cells. This cytokine stimulates macrophages, natural killer cells, and T-cell proliferation, which mediate the cellular immune response (Wilson *et al.*, 1996). IL-2 has also been implicated in the stimulation of osteoclast activity in bone resorption and has been suggested to play an active role in the pathogenesis of periodontal disease (Scarel-Caminaga *et al.*, 2002). IL-6 regulates immune responses at inflammatory sites and has autocrine/paracrine activities that stimulate the osteoclast formation and bone resorbing activity of preformed osteoclasts (Okada *et al.*, 1997). IL-8 plays a key role in recruiting and activating neutrophils

during inflammation. It is secreted mainly by monocytes and is important for regulating alveolar bone resorption during tooth movement by acting at an early stage in the inflammatory response (Baggiolini *et al.*, 1989).

Mini-implants are increasingly used as orthodontic anchorage. They provide stable bony anchorage and overcome problems due to anchorage loss during space closure, which usually occurs with traditional anchorage techniques (Park *et al.*, 2004).

Peri-implantitis is clinically characterized by increased probing depth, pain, and/or radiographic bone loss, which may cause implant failure (Meffert, 1992). Many studies on dental implants have reported that an increase in proinflammatory cytokine levels in peri-implant crevicular fluid causes peri-implantitis (Kao *et al.*, 1995; Panagakos *et al.*, 1996; Aboyoussef *et al.*, 1998). Shaama (2005) and Nowzari *et al.* (2008) found that IL-6 and IL-8 levels were significantly higher in patients with failing implants than in those with healthy implants.

Based on this information, the aims of this study were to measure the levels of proinflammatory cytokine, IL-2, IL-6, and IL-8, around healthy miniscrews during 3 months of canine distalization and to compare the results with the cytokine levels around healthy teeth.

Subjects and methods

Sixteen patients (eight males and eight females; mean age, 16.6 ± 2.4 years) who required extraction of their bilateral upper first premolars for orthodontic purposes were included in this study. The inclusion criteria were a healthy systemic condition, no use of anti-inflammatory drugs in the 6 months preceding the start of the study, and no radiographic evidence of periodontal bone loss after a full-mouth radiographic periapical examination. Patients were also evaluated for periodontal health using the plaque index, gingival index, pocket depth, and bleeding on probing. Informed consent was obtained from all patients and/or the parents of those under 18 years of age.

Thirty-two miniscrew implants were inserted in gingiva with no inflammation and no peri-implant pockets greater than 3 mm, which were accepted as healthy according to the criteria of [Aboyoussef et al. \(1998\)](#), similar to the study of [Sari and Uçar \(2007\)](#).

Clinical procedures

Initially a fixed, preadjusted edgewise appliance with 0.018 inch slots was inserted, and a 0.014 inch nickel–titanium (NiTi) archwire was placed for levelling. After alignment of the maxillary anterior teeth, a 0.016×0.022 inch stainless steel archwire with molar toe–ins and tip–back bends was placed, and the second maxillary premolars and first molars were ligated together before beginning distal movement of the canines.

Thirty-two miniscrew implants (length 12 mm and diameter 1.6 mm; Anchor Plus, Buk-Gu, Gwangju, Korea) were placed bilaterally into the inter-radicular bone between the maxillary second premolars and first molars, as described by [Park et al. \(2001, 2002\)](#). Two weeks after placement, distalization of the maxillary canines commenced with 150 g force delivered by 7 mm Sentalloy closed coil spring (GAC International, Bohemia, New York, USA) between the miniscrew and canine (Figure 1).

The miniscrew group consisted of a total of 32 miniscrew implants placed bilaterally in 16 patients in whom the upper first premolars were extracted. The treatment group comprised 32 upper bilateral canines and the control group 32 upper bilateral second premolars.

Collection of gingival crevicular fluid and peri-miniscrew implant crevicular fluid samples

Gingival crevicular fluid (GCF) samples were obtained from the maxillary canines (treatment) and the maxillary second premolar teeth (controls) with paper strips (Periopaper®; Pro Flow, Amityville, New York, USA) using the method described by [Rudin et al. \(1970\)](#). Peri-miniscrew implant crevicular fluid (PMICF) samples were also collected with paper strips. The samples were obtained over a 3 month time



Figure 1 Distalization a using a nickel-titanium closed coil spring inserted between the miniscrew and canine.

period according to the following schedule: T1 (baseline), 2 weeks after the insertion of miniscrews and before activation: T2, T3, and T4 at 1, 24, and 48 hours; T5 and T6 on days 7 and 21, respectively, and T7, 3 months after activation of the closed coil springs.

The samples were collected during the morning. Plaque was removed without touching the gingiva to minimize contamination of the paper strips. The site was gently washed with water, isolated with cotton rolls (to eliminate contamination from saliva), and gently dried with an air syringe. GCF and PMICF were collected as in the study of [Sari and Uçar \(2007\)](#) from the mesiobuccal aspects of the miniscrew implants. Two strips of filter paper were used, and the samples were placed in Eppendorf tubes. GCF samples were also obtained from the distobuccal sites of the maxillary canines. Two filter papers, which were placed in additional Eppendorf tubes. Similarly, two filter papers were used for the control teeth. The first strip was inserted into the base of the pocket for 30 seconds and after a 1 minute interval, a second strip was inserted for 30 seconds. A Periotron 8000 (Ora Flow Inc., Plainview, New York, USA) was used to determine the volume of GCF and PMICF. The paper strips were stored in sterile tubes at -20°C until the start of the experiment. Saliva and blood contaminated samples were discarded. GCF and PMICF samples were taken before all other clinical examinations were performed to prevent an increase in fluid volume.

Before examining the GCF and PMICF samples, 1000 μl sterile NaCl (9 mg/ml) was added to the paper strips, and the samples were centrifuged at 3000 g at 5°C for 20 minutes ([Tzannetou et al., 1998](#)). An immunoassay kit was used to measure IL-2, IL-6, and IL-8 concentrations (Immulite; Diagnostic Products, Los Angeles, California, USA). IL-2, IL-6, and IL-8 free non-human buffer matrix was used to manually dilute the samples.

The amounts of IL-2, IL-6, and IL-8 in each sample were compared with standard curves for these interleukins, which

showed a direct relationship between optical density and cytokine concentration.

Statistical evaluation

Normal distributions were evaluated using the Kolmogorov–Smirnov test, and homogeneity was assessed with the Levene test. A paired sample *t*-test for within-group differences was used. One-way analysis of variance was used for between-groups comparison, and Dunnett's *t* and Tukey's honestly significant difference tests for between-group multiple comparison. All data were analysed using the Statistical Package for Social Science version 15.0 (SPSS Inc., Chicago, Illinois, USA). $P < 0.05$ was considered as statistically significant.

Results

A significant increase in IL-2 level occurred only at T3 ($P < 0.01$; Table 1), with no significant differences between the groups ($P = 0.234$ to 0.999). No differences in the IL-6 level were detected in any group in the within-group comparison at T7 (Table 1). For the between-group evaluation, IL-6 was significantly different between the treatment miniscrew groups ($P < 0.05$) and the control miniscrew group ($P < 0.05$) at T7. There was no significant change at any other time point ($P = 0.260$ to 0.999).

IL-8 level showed significant increases in the treatment group at T2 ($P < 0.05$), T3 ($P < 0.01$), and T4 ($P < 0.01$) and at T3 ($P < 0.05$) and T4 ($P < 0.01$) in the miniscrew group (Table 1). No significant changes were observed between the groups at any time point ($P = 0.065$ to 1.000).

Discussion

Anchorage control is a fundamental concept in orthodontic treatment. Therefore, treatment objectives should consider the effectiveness of orthodontic anchorage for the most efficient correction of a patient's dental or skeletal malocclusion (Nanda and Kuhlberg, 1997). Miniscrew implants that have increasingly been used for anchorage control in orthodontic treatment for non-compliant patients have many advantages, such as ease of placement and removal, small size, and low cost (Kanomi, 1997; Park and Kwon, 2004).

Proinflammatory cytokines play important roles in bone and root resorption (Başaran *et al.*, 2006). Some studies (Kao *et al.*, 1995; Shaama, 2005) have shown that significant increases in proinflammatory cytokines cause dental implant failure, but no study has reported on the role of IL-2, IL-6, and IL-8 in this process of failure of miniscrew implants. Thus, the aim of the present research was to measure the levels of these proinflammatory cytokines around miniscrews. The amount of IL-2, IL-6, and IL-8 around miniscrews with force applied to the maxillary canines (treatment) or no force applied to maxillary second

Table 1 Mean interleukin levels (picograms per microlitre) and standard deviations (SDs) at all time points and within-group comparisons of the baseline value with values at different time points.

Group, <i>n</i> = 32	T1 (baseline), mean ± SD	T2 (1 hour), mean ± SD	<i>P</i> (T1–T2)	T3 (24 hours), mean ± SD	<i>P</i> (T1–T3)	T4 (48 hours), mean ± SD	<i>P</i> (T1–T4)	T5 (7 days), mean ± SD	<i>P</i> (T1–T5)	T6 (21 days), mean ± SD	<i>P</i> (T1–T6)	T7 (3 months), mean ± SD	<i>P</i> (T1–T7)
Interleukin-2													
Treatment	69.94 ± 31.22	72.01 ± 30.46	0.777	78.01 ± 27.78	**	78.48 ± 30.02	0.089	77.51 ± 17.91	0.114	78.08 ± 35.11	0.111	74.01 ± 29.81	0.058
Control	67.14 ± 20.72	68.08 ± 21.05	0.764	70.01 ± 31.24	0.693	71.01 ± 29.83	0.586	70.01 ± 30.97	0.690	68.05 ± 25.37	0.879	65.97 ± 26.42	0.847
Miniscrew	65.20 ± 20.73	68.33 ± 21.69	0.238	74.19 ± 30.36	0.219	72.66 ± 21.90	0.175	70.58 ± 30.84	0.454	70.32 ± 16.04	0.330	67.43 ± 25.17	0.705
Interleukin-6													
Treatment	2.95 ± 2.66	3.05 ± 1.62	0.735	3.15 ± 1.83	0.754	3.20 ± 1.82	0.685	3.04 ± 0.99	0.853	2.99 ± 1.15	0.951	2.95 ± 1.04	1.000
Control	2.80 ± 0.57	2.89 ± 1.98	0.808	2.92 ± 1.15	0.653	2.92 ± 1.03	0.351	2.78 ± 0.75	0.846	2.69 ± 2.57	0.823	2.62 ± 1.20	0.510
Miniscrew	3.01 ± 0.82	3.23 ± 0.78	0.262	3.36 ± 0.97	0.135	3.42 ± 1.19	0.085	3.05 ± 1.49	0.873	3.02 ± 0.76	0.956	3.25 ± 0.71	0.209
Interleukin-8													
Treatment	75.02 ± 14.59	80.81 ± 13.68	*	84.36 ± 8.79	**	85.18 ± 9.99	**	81.31 ± 16.82	0.107	80.40 ± 11.13	0.069	75.13 ± 13.24	0.967
Control	76.61 ± 14.78	78.17 ± 16.97	0.655	78.56 ± 17.10	0.426	79.10 ± 13.51	0.378	78.14 ± 10.70	0.621	76.88 ± 14.32	0.894	75.13 ± 13.63	0.608
Miniscrew	77.84 ± 12.59	78.90 ± 8.32	0.573	83.52 ± 14.34	*	84.44 ± 10.37	**	81.62 ± 12.58	0.191	79.75 ± 13.94	0.403	78.01 ± 12.12	0.931

* $P < 0.01$, ** $P < 0.01$.

premolars (control) was compared. As Serra *et al.* (2003) found that age and gender did not increase enzymatic activity, these factors were not considered in the present study.

IL-2 has been implicated in the stimulation of osteoclast activity during bone resorption (Ries *et al.*, 1989), and serum IL-2 levels are elevated in patients with periodontitis when compared with those without periodontitis (McFarlane and Meikle, 1991). IL-2 levels were measured because IL-2 has been suggested to be a useful marker of inflammatory activity due to its biological properties (John *et al.*, 1998). At the end of the study, a significant increase in IL-2 levels during canine distalization was found at T3. Başaran *et al.* (2006) measured IL-2 level around canines during orthodontic treatment and found increases relative to baseline. The findings of the present study were comparable with these results; IL-2 level around miniscrews did not change significantly. However, in the initial days, a slight increase in IL-2 levels was observed. This finding is comparable with that of Campos *et al.* (2005) who studied early implant failures.

IL-6 is a multi-functional cytokine, and increased levels are involved in bone destruction. In studies performed during orthodontic treatment (Davidovitch *et al.*, 1988; Alhashimi *et al.*, 2001), and in a dental implant study (Shaama, 2005), IL-6 levels increased from the first hour and decreased after 7 and 10 days. In the current research, no statistically significant increase in IL-6 levels among the three groups was found during any period; however, an increase in IL-6 was observed around the canines and miniscrews from T2 to T5. This result was compatible with the findings of the above studies, although Başaran *et al.* (2006) reported no increase in IL-6 levels during canine distalization.

The various activities of IL-8 indicate that this cytokine plays a major role in mediating inflammatory responses (Garrett and Mundy, 1989). Sfakianakis *et al.* (2002) reported the precise location of IL-8 receptors in periodontitis and non-inflamed human gingivae and suggested that IL-8 plays multifunctional roles in the pathogenesis of periodontal disease. Orthodontic forces evoke changes in IL-8 levels (Baloş Tuncer *et al.*, 2005). Those authors reported significant increases in IL-8 during canine distalization beginning from the first hour, which reached a maximum on day 6. Başaran *et al.* (2006) found increases in IL-8 levels at days 7 and 21 during canine distalization. In the present study, IL-8 levels increased significantly in both the miniscrew and the canine groups at T3 and T4. This result was compatible with the studies of Baloş Tuncer *et al.* (2005), Shaama (2005), and Başaran *et al.* (2006).

The increases in cytokine levels in the current study were similar for canines and around miniscrews; they occurred in the first hours and indicated an acute reaction. Thus, as mediators are effective in the early stages of inflammation, the amount of initial force is important. This result is

compatible with the findings of Alhashimi *et al.* (2001) and Baloş Tuncer *et al.* (2005). Shaama (2005) also reported that IL-6 and IL-8 levels in implant crevicular fluid increased from day 1 but decreased by day 10.

When between-group comparisons were examined, IL-6 and IL-8 levels around miniscrews were higher than the cytokine levels around the canine at T1 and T6. Moreover, the IL-6 level around miniscrews increased significantly at T7 relative to both the control and treatment groups. This was compatible with the report of Nowzari *et al.* (2008) who found higher IL-6 and IL-8 levels around healthy implants than teeth. However, in the current research, no significant differences were observed in IL-2 levels between the miniscrews and teeth.

Shaama (2005) reported that implant material plays an important role in tissue reaction. Ions released from dental implants can stimulate peripheral blood mononuclear cells to produce IL-1 β and tumour necrosis factor- α *in vitro* (Rogers *et al.*, 2002). Commercially pure titanium and titanium alloys have also been associated with the production of IL-6 and IL-18 (Spyrou *et al.*, 2000). Thus, the different interleukin levels around miniscrews were attributed to the implant material.

Conclusions

The level of miniscrew loading should be controlled to avoid cytokine increase and hence screw loosening. Care should be taken to mitigate increases in proinflammatory cytokine levels around miniscrews by controlling the initial force.

References

- Abouyoussef H, Carter C, Jandiski J J 1998 Detection of prostaglandin E₂ and matrix metalloproteinases in implant crevicular fluid. *International Journal of Oral and Maxillofacial Implants* 13: 689–696
- Alhashimi N, Frithiof L, Brudvik P, Bakhiet M 2001 Orthodontic tooth movement and *de novo* synthesis of proinflammatory cytokines. *American Journal of Orthodontics and Dentofacial Orthopedics* 119: 307–312
- Baggiolini M, Walz A, Kunkel S L 1989 Neutrophil-activating peptide-1/IL-8, a novel cytokine that activates neutrophil. *Journal of Clinical Investigation* 84: 1045–1049
- Baloş Tuncer B *et al.* 2005 Levels of interleukin-8 during tooth movement. *Angle Orthodontist* 75: 631–636
- Başaran G, Özer T, Acun Kaya F, Hamamcı O 2006 Interleukins 2, 6, and 8 levels in human gingival sulcus during orthodontic treatment. *American Journal of Orthodontics and Dentofacial Orthopedics* 130: 7.e1–e6
- Campos M I G, Godoy dos Santos M C L, Trevilatto P C, Scarel-Caminaga R M, Bezerra F J, Line S R P 2005 Interleukin-2 and interleukin-6 gene promoter polymorphisms, and early failure of dental implants. *Implant Dentistry* 14: 391–398
- Davidovitch Z, Nicolay O, Ngan P W, Shanfeld J L 1988 Neurotransmitters, cytokines and the control of alveolar bone remodeling in orthodontics. *Dental Clinics of North America* 32: 411–435
- Garrett I R, Mundy G R 1989 Relationship between interleukin-1 and prostoglandins in resorbing neonatal calvariae. *Journal of Bone and Mineral Research* 4: 789–794

- Graves D T, Cochran D 2003 The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. *Journal of Periodontology* 74: 391–401
- John S *et al.* 1998 Two novel biallelic polymorphism in the IL-2 gene. *European Journal of Immunogenetics* 25: 419–420
- Kanomi R 1997 Mini-implant for orthodontic anchorage. *Journal of Clinical Orthodontics* 31: 763–767
- Kao R T, Curtis D A, Richards D W, Preble J 1995 Increased interleukin-1 β in the crevicular fluid of diseased implants. *International Journal of Oral and Maxillofacial Implants* 10: 696–701
- McFarlane C G, Meikle M C 1991 Interleukin-2, interleukin-2 receptor and interleukin-4 levels are elevated in the sera of patients with periodontal disease. *Journal of Periodontal Research* 26: 402–408
- Meffert R M 1992 Treatment of the ailing, failing implant. *Journal of the California Dental Association* 20: 42–45
- Nanda R, Kuhlberg A 1997 Biomechanical basis of extraction space closure. In: Nanda R (ed). *Biomechanics in clinical orthodontics*. Saunders, Philadelphia, pp. 156–187
- Nowzari H, Botero J E, De Giacomo M, Villacres M C, Rich S K 2008 Microbiology and cytokine levels around healthy dental implants and teeth. *Clinical Implant Dentistry* 10: 166–173
- Okada N *et al.* 1997 Interleukin-6 production in human fibroblasts derived from periodontal tissues is differentially regulated by cytokines and a glucocorticoid. *Journal of Periodontal Research* 32: 559–569
- Panagakos F S, Aboyoussef H, Dondero R, Jandinski J J 1996 Detection and measurement of inflammatory cytokines in implant crevicular fluid: a pilot study. *International Journal of Oral and Maxillofacial Implants* 196: 794–799
- Park H S, Bae S M, Kyung H M, Sung J H 2001 Micro-implant anchorage for treatment of skeletal Class I bialveolar protrusion. *Journal of Clinical Orthodontics* 35: 417–422
- Park H S, Kwon T G 2004 Sliding mechanics with microcrew implant anchorage. *Angle Orthodontist* 74: 703–710
- Park H S, Kwon T G, Sung J H 2004 Nonextraction treatment with microcrew implants. *Angle Orthodontist* 74: 539–549
- Park H S, Kyung H M, Sung J H 2002 Simple method of molar uprighting with micro-implant anchorage. *Journal of Clinical Orthodontics* 36: 592–596
- Ries W R, Seeds M C, Key L L 1989 Interleukin-2 stimulates osteoclastic activity: increased acid production and radioactive calcium release. *Journal of Periodontal Research* 24: 242–246
- Rogers M *et al.* 2002 Do interleukin-1 polymorphisms predict the development of periodontitis or the success of dental implants? *Journal of Periodontal Research* 37: 37–41
- Rudin H J, Overdizk H F, Rateitschack K H 1970 Correlations between sulcus fluid rate and clinical and histological inflammation of the marginal gingival. *Helvetica Odontologia Acta* 14: 21–26
- Sarı E, Uçar C 2007 Interleukin 1 β levels around microcrew implants during orthodontic tooth movement. *Angle Orthodontist* 77: 1073–1078
- Scarel-Caminaga R M, Trevilatto P C, Souza A P, Brito R B Jr, Line S R P 2002 Investigation of an IL-2 polymorphism in patients with different levels of chronic periodontitis. *Journal of Clinical Periodontology* 29: 587–591
- Serra E *et al.* 2003 Lactate dehydrogenase activity in gingival crevicular fluid during orthodontic treatment. *American Journal of Orthodontics and Dentofacial Orthopedics* 124: 206–211
- Sfakianakis A, Barr C E, Kreutzer D L 2002 Localization of the chemokine interleukin-8 and interleukin-8 receptors in human gingiva and cultured gingival keratinocytes. *Journal of Periodontal Research* 37: 154–160
- Shaama F A 2005 An *in vitro* comparison of implant materials, cell attachment, cytokine and osteocalcin production. *West Indian Medical Journal* 54: 250–256
- Spyrou P, Papaioannou S, Hampson G, Brady K, Palmer R, McDonald F 2000 Cytokine release by osteoblast-like cells cultured on implant discs of varying alloy compositions. *Clinical Oral Implants Research* 13: 623–630
- Stoycheva M S, Murdjeva M A 2005 Correlation between serum levels of interleukin 1- β , interleukin 1-ra, interleukin-6, interleukin 10, interleukin 12, tumor necrosis factor and interferon- γ with some clinical and laboratory parameters in patients with salmonellosis. *Biotechnology and Biotechnological Equipment* 19: 143–146
- Tzannetou S, Efstratiadis S, Nicolay O, Grbic J, Lamster I 1998 Interleukin 1 β and β -glucuronidase in gingival crevicular fluid from molars during rapid palatal expansion. *American Journal of Orthodontics and Dentofacial Orthopedics* 114: 686–696
- Wilson M, Reddi K, Henderson B 1996 Cytokine-inducing components of periodontopathogenic bacteria. *Journal of Periodontal Research* 31: 393–407

Copyright of European Journal of Orthodontics is the property of Oxford University Press / UK and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.