Ultrastructural muscle damage in young vs. older men after high-volume, heavy-resistance strength training

STEPHEN M. ROTH,1 GREGORY F. MARTEL,1,2 FREDERICK M. IVEY,1 JEFFREY T. LEMMER,1 BRIAN L. TRACY,1 DIANE E. HURLBUT,1 E. JEFFREY METTER,3 BEN F. HURLEY,1 AND MARC A. ROGERS1

1Department of Kinesiology, University of Maryland College Park, College Park, 20742;
2Department of Physical Therapy, University of Maryland Eastern Shore, Princess Anne, 21853; and
3Gerontology Research Center, National Institute on Aging, Baltimore, Maryland 21224

Ultrastructural muscle damage in young vs. older men after high-volume, heavy-resistance strength training. J. Appl. Physiol. 86(6): 1833–1840, 1999.—This study assessed ultrastructural muscle damage in young (20–30 yr old) vs. older (65–75 yr old) men after heavy-resistance strength training (HRST). Seven young and eight older subjects completed 9 wk of unilateral leg extension HRST. Five sets of 5–20 repetitions were performed 3 days/wk with variable resistance designed to subject the muscle to near-maximal loads during every repetition. Biopsies were taken from the vastus lateralis of both legs, and muscle damage was quantified via electron microscopy. Training resulted in a 27% strength increase in both groups (P < 0.05). In biopsies before training in the trained leg and in all biopsies from untrained leg, 0–3% of muscle fibers exhibited muscle damage in both groups (P = not significant). After HRST, 7 and 6% of fibers in the trained leg exhibited damage in the young and older men, respectively (P < 0.05, no significant group differences). Myofibrillar damage was primarily focal, confined to one to two sarcomeres. Young and older men appear to exhibit similar levels of muscle damage at baseline and after chronic HRST.

aging; muscle fiber hyper-contraction; muscle injury; regeneration; resistance training

AGING RESULTS in a progressive loss of strength and muscle mass (28) that is associated with a decline in functional capacity (15). Heavy-resistance strength training (HRST) can lead to substantial strength and muscle mass increases (3, 11) that result in improved functional capacity even in the very old (7). However, studies that have examined the chronic effects of strength training, but older individuals were not examined. Hurlay et al. (14) reported lower serum concentrations of creatine kinase (an indicator of muscle cell disruption) in older men after 16 wk of strength training, compared with before-training values, but muscle ultrastructure was not assessed. Brown (4) reported no increase in histological muscle damage after strength training in older rats. We are unaware of any studies that have assessed ultrastructural muscle damage in older human muscle after chronic HRST. Thus the purpose of the present investigation was to compare the effects of 9 wk of high-volume HRST on ultrastructural muscle damage of the vastus lateralis muscle in both young and older men.

METHODS

Subject selection. Twenty healthy subjects completed all aspects of this study. The subjects consisted of 10 young men between 20 and 30 yr and 10 older men between 65 and 75 yr. All subjects were screened by a physician, who performed a medical history, physical examination, and graded maximal-exercise test. All subjects were nonsmokers, free of significant cardiovascular, metabolic, or musculoskeletal disorders. Individuals enrolled in the study had not participated in a regular exercise program for at least 6 mo before their recruitment.
After all methods and procedures were explained, the subjects read and signed a written consent form for the project, which had been approved by the Institutional Review Board of the University of Maryland, College Park. All subjects were reminded throughout the study not to alter their regular physical activity levels or dietary habits for the duration of the investigation.

Body composition. Body composition was assessed before and after training by using a Lunar DPX dual-energy X-ray absorptiometer (Lunar software v. 1.22, Lunar), as previously described (27). Subjects were instructed not to eat or drink for at least 8 h before their morning scan. A calibration standard was scanned daily, and measurement accuracy was maintained by using a water/oil phantom of known proportions. The coefficient of variation for repeated measurements was <1.0%. Body weight was measured weekly by using a medical beam scale, and subjects were encouraged to maintain their before-training body weight. Strength testing. Muscle strength was assessed in both quadriceps unilaterally via a one-repetition maximum (1RM) test for which the Keiser K-300 pneumatic variable-resistance leg-extension machine was used. Three training sessions were conducted with the use of light resistance before the strength testing to familiarize the subjects with the equipment, training protocol, and proper exercise technique.

In addition, this familiarization procedure was designed to help control for the large initial strength increase due to motor learning and to help prevent injuries. After the familiarization sessions and before the HRST sessions began, concentric knee-extensor strength was assessed in both legs via the 1RM test, which was repeated after the training. Before each test, the subjects performed a 3-min warm-up on a stationary cycle and static stretching of the quadriceps. Subjects were positioned on the knee-extension machine with a pelvis strap to stabilize other muscle groups, and the nontesting leg was placed in front of the contralateral leg-extension pad to prevent voluntary contraction. After a few warm-up repetitions, a resistance was chosen that was thought to be slightly below the concentric 1RM, and the subject performed one repetition. Increases in resistance between trials were adjusted to minimize the total number of trials (6–8) required before the true 1RM was obtained and to keep the number of trials similar before and after training. Rest periods of ~60 s were allowed between trials. The same investigator measured 1RM strength before and after training, and all testing procedures were standardized based on specific seat and body positions. To determine the initial resistance for the first regular HRST session, the five-repetition maximum (5RM) was also determined by using the same procedure.

HRST protocol. Each training session was preceded by a 3-min warm-up on a stationary cycle, followed by 5–10 min of static stretching of the quadriceps. The training protocol consisted of 9 wk of unilateral HRST of the knee extensors of the dominant leg, with the nondominant leg serving as a control. During the training sessions, the untrained leg was placed in front of the contralateral leg-extension pad to prevent voluntary contraction of that muscle, which was verified by investigator’s observation during each training session. The Keiser K-300 leg-extension machine, which allows for changing the resistance easily without interrupting the cadence of the exercise, was used throughout the training program. The training program consisted of five sets of high-volume (55 total repetitions), heavy-resistive exercise performed 3 days/wk. The resistance for each set was based on the subject’s 5RM. After performing a first set of five repetitions at 50% of the original 1RM for a warm-up, the subject performed a second set of five repetitions at the 5RM resistance after a 30-s rest period. The third set consisted of 10 repetitions, with the first four or five repetitions at the current 5RM value, then the resistance was lowered just enough to complete one or two more repetitions before the subject reached fatigue. The process was repeated until a total of 10, 15, and 20 repetitions were completed for the third, fourth, and fifth sets, respectively. The third, fourth, and fifth sets were preceded by rest periods lasting a minimum of 90, 150, and 180 s, respectively. This protocol demanded a near-maximal effort on every repetition throughout each set. The concentric phase of each repetition was performed in ~1–2 s and the eccentric phase in 2–3 s. An exercise specialist supervised the exercise sessions of every subject at every training session to verify compliance with the training protocol. Increases in resistance occurred throughout the training program and were based on the subject’s ability to perform the 5RM of the first and subsequent sets.

Muscle tissue sampling. Bilateral muscle biopsies were taken from the vastus lateralis muscle of a subject before and after the HRST protocol by using the percutaneous biopsy technique (1). The initial sampling site determined for each subject was 16 cm from the proximal border of the patella at the midline of the quadriceps. The muscle sample was obtained with suction by using a 5-mm Bergstrom biopsy needle. The biopsy sample taken after training was obtained at a new site 2.5 mm proximal and lateral to the original incision, with the biopsy needle directed into approximately the same muscle position as the first biopsy. The before-training biopsy occurred ~1 wk before the familiarization sessions and 2 wk before the start of the training program. The after-training biopsy occurred 24–48 h after the last training session.

Muscle fixation and analysis. The muscle sample (~50–70 mg) was placed immediately on an ice-chilled watch glass and dissected of all visible blood and adipose and connective tissues. The sample was minced for fixation into eight to ten 0.5- to 1.0-mm cubes. The cubes were placed into a 10-ml glass vial containing a 2% solution of gluteraldehyde in 0.12 M phosphate buffer (Millonig’s buffer). The samples were fixed at room temperature for 1 h and then refrigerated (5–10°C) until postfixation. The minced tissue samples were postfixed for 2 h in 1.0% osmium tetroxide. After the postfixation, the samples were stained for 60 min in 2% uranyl acetate to induce a positive contrast. The samples were subsequently dehydrated by using a graded series of ethanol washes (50, 75, 95, and 100%). The tissue samples were longitudinally oriented and embedded in epoxy resin (Spurr’s). Five sample blocks were obtained from each muscle biopsy sample. The sample blocks from each biopsy were trimmed of excess plastic resin and sectioned by using a glass knife for thick sections and a diamond knife (Diatome) for thin sections. Thick sections, 0.8–1.0 µm, were cut initially and stained by using toluidine blue or methylene blue/azure II and mounted on slides for assessment of fiber orientation. Thin sections were then cut (60–70 nm, gold to silver) and viewed at 10,000–40,000× by using a transmission electron microscope. Thin sections were placed on 75 × 300 copper grids. Two grids were obtained from each sample, and the sections on each grid were stained with 2% uranyl acetate and 0.1% lead citrate for positive contrast. Preparated grids were viewed on a Zeiss EM 10 CA electron microscope operated at 80 kV. A representative section on each grid was viewed at X2,000–10,000, and micrographs were taken of each fiber. Muscle damage was quantified initially by using images directly from the electron microscope and subsequently confirmed via prepared micrographs. The primary investigator was blind to both the treatment group and the time point during the analysis. A blinded
second investigator repeated the analysis separately by using
the prepared micrographs, and reliability was calculated
from the results (Pearson’s $r = 0.95$).

Quantification of muscle damage. Each viable muscle fiber
was analyzed for ultrastructural muscle damage. Hypercon-
trated fibers (Fig. 1) were excluded from the analysis, as the
cause of the associated damage was unable to be determined
(see DISCUSSION for detailed explanation). A viable muscle
fiber was defined as a transverse or longitudinally oriented
fiber with a minimum visible length of 200 µm with minimal
hypercontraction (Fig. 2 represents a normal skeletal muscle
fiber). Initially, 30–40 fibers were assessed for each subject,
but only 10–30 fibers were analyzed for muscle damage for
each subject per time point, due to the elimination of hypercon-
trated fibers. Each fiber was analyzed individually for the
extent of damage, and all fibers per subject were assessed for
the extent of fiber disruption. The quantification of muscle
damage was performed based on the work of Newham et al.
(21) and Gibala et al. (12). A disrupted fiber was considered
any fiber containing apparent disturbances in the normal
myofibrillar banding pattern. Specifically, fibers exhibiting
Z-line streaming or M-band disruption, as well as disruption
of the myofilament structure within sarcomeres, were classi-
fied as damaged. An area of disruption occupying one to two
adjacent myofibrils and/or one to two continuous sarcomeres
was classified as a “focal” disruption (21). An area of disruption
encompassing 3–10 adjacent myofibrils and/or 3–10
continuous sarcomeres was designated as “moderate” disruption,
and an area of disruption covering >10 adjacent myofi-

![Fig. 1. Electron micrograph of a hypercontracted skeletal muscle
fiber. Notice the maintenance of M band and myofilament structure
despite severe contraction (magnification ×16,000).](image1)

![Fig. 2. Electron micrograph of a normal human skeletal muscle fiber
(magnification ×16,000).](image2)

Statistical analysis. Because of extensive hypercontraction
of several muscle samples, muscle samples from three young
and two older subjects were eliminated from the analysis,
leaving seven young and eight older subjects for statistical
analysis. Furthermore, no fibers exhibited extreme muscle
damage, and thus all damaged fibers (predominantly catego-
rized as focal damage) were pooled for statistical analysis.
Ultrastructural damage and strength test data were ana-
alyzed by using a two-factor (2 × 2; time × group) repeated-
measures ANOVA. Group differences were then determined
by using a Bonferroni post hoc analysis. Statistical signifi-
cance for all ANOVA analyses was accepted at $P < 0.05$,
whereas the significance level associated with the Bonferroni
post hoc analysis for two groups was $P < 0.025$. All data are
reported as means ± SD.

RESULTS

Physical characteristics and 1RM strength values. Subject
characteristics are listed in Table 1. Body weight remained stable in
both the young and older men throughout the 9 wk, and only age
and maximal oxygen uptake values were significantly different
between the groups (Table 1). All subjects completed a
minimum of 27 supervised exercise sessions over ~9 wk. Before training, there was no significant difference
in 1RM strength for the knee extensors of the young
men compared with the older men. In addition, no
significant strength differences existed between the
trained and untrained limbs before training in either group. After 9 wk of unilateral HRST of the dominant knee extensors, both the young and older men increased strength significantly (26 and 28%, respectively, \( P < 0.05 \)) (Table 2). The strength of the untrained leg also increased in both the young and older men (6 and 13%, respectively); however, only the older men showed a significant increase in strength of the untrained leg (\( P < 0.05 \)).

Muscle fiber damage. As shown in Fig. 3, before training, no muscle fibers from the trained leg of the older men exhibited myofibrillar damage (all muscle damage categories pooled), whereas the young men exhibited a small percentage (1.2 ± 3.1%) of damaged fibers (\( P \) not significant (NS)). In the young men, the damage was categorized as focal according to the established criteria (12, 21). Muscle fibers obtained from the untrained legs before training also exhibited focal damage. In the older men, 1.6 ± 3.2% of fibers exhibited muscle damage compared with 2.5 ± 4.5% of fibers for the young men (\( P = \) NS).

After 9 wk of HRST, ultrastructural muscle damage increased significantly in the young (7.1 ± 9.3% of fibers exhibited damage) and older men (5.6 ± 6.2% of fibers exhibited damage) (\( P < 0.05 \)); however, no group differences existed (\( P = \) NS) (Fig. 3). Analysis of the micrographs from the untrained leg after the training protocol revealed that 2.8 ± 5.6 and 0% of fibers exhibited myofibrillar damage in the older and young men, respectively. These values were not significantly different from before training.

Figure 2 is a representative micrograph of a normal human skeletal muscle fiber. The damage in the muscle fibers was primarily categorized as focal (damage limited to 1-2 continuous or adjacent sarcomeres). No fibers were found with extreme damage, and fibers exhibiting moderate damage (3-10 sarcomeres affected) were found mainly in the older men after the training protocol (33% of damaged fibers in the older men after training). Most of the focal damage consisted of "frayed" or smeared sarcomeres, with myofibrils showing a disruption from their normal banding pattern (Fig. 4). Moderate damage generally consisted of several sarcomeres with a smeared appearance and

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Young Men (n = 7)</th>
<th>Older Men (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Training</td>
<td>After Training</td>
</tr>
<tr>
<td>Age, yr</td>
<td>25 ± 3*</td>
<td>69 ± 3</td>
</tr>
<tr>
<td>Height, cm</td>
<td>180.4 ± 10.7</td>
<td>173.3 ± 4.3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>85.2 ± 18.3</td>
<td>84.3 ± 9.4</td>
</tr>
<tr>
<td>%Fat</td>
<td>25.5 ± 8.1</td>
<td>29.6 ± 5.6</td>
</tr>
<tr>
<td>( V_{\text{O}}2_{\text{max}}, \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} )</td>
<td>43.3 ± 3.7*</td>
<td>23.5 ± 4.7</td>
</tr>
</tbody>
</table>

Values are means ± SD. \( V_{\text{O}}2_{\text{max}} \), maximal \( O_2 \) uptake. *Significant difference, \( P < 0.05 \).

### Table 2. 1RM strength values for young and older men after 9 wk of unilateral heavy-resistance strength training

<table>
<thead>
<tr>
<th>Leg</th>
<th>Young Men (n = 7)</th>
<th>Older Men (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Training</td>
<td>After Training</td>
</tr>
<tr>
<td>T</td>
<td>79.4 ± 21.1</td>
<td>99.8 ± 22.7*</td>
</tr>
<tr>
<td>UT</td>
<td>87.6 ± 21.2</td>
<td>93.7 ± 26.5</td>
</tr>
</tbody>
</table>

Values are means ± SD in kg. T, trained leg; UT, untrained leg; 1RM, 1-repetition maximum. *Significantly different than before training, \( P < 0.05 \).

Fig. 3. Percent of muscle fibers exhibiting ultrastructural muscle damage for both trained (T) and untrained (UT) legs. Before, before training; After, after training. *\( P < 0.05 \) vs. T Before.

Fig. 4. Electron micrograph of a longitudinally oriented skeletal muscle fiber exhibiting focal muscle damage occupying a single sarcomere and associated Z disks (magnification ×16,000).
damaged Z disks (Fig. 5). Occasionally, Z disks alone would show indications of damage (Fig. 6). Although not quantified, myofibrillar splitting was commonly exhibited throughout both trained and untrained biopsies of both groups, with no apparent relation to myofibrillar damage (Figs. 4 and 6). Several fibers obtained from both groups after the training protocol exhibited separation of myofibrils (Fig. 7). This was rarely observed in before-training or untrained muscle samples.

**DISCUSSION**

The present study assessed ultrastructural muscle damage in young and older men both before and after a prolonged HRST program. Past research has indicated that older skeletal muscle may be more susceptible to muscle damage associated with strenuous exercise than that of young muscle (2, 17, 18). After 9 wk of high-volume HRST, increases in ultrastructural damage in the vastus lateralis muscle were similar in young and older men in the present study. In older rats, the introduction of a strength training program did not result in exercise-related muscle damage, but no ultrastructural assessment was made (4). Thus resistance exercise could be introduced to older rats without risk of excessive histological muscle damage (4). The present investigation is the first we are aware of supporting a similar conclusion for older humans.

Analysis of muscle damage in the biopsy specimens from the trained leg of both groups before HRST indicated no physiologically significant level of damage in either group and no significant difference in the degree of muscle damage between groups. Similarly, the muscle samples from the untrained legs did not exhibit a significant degree of muscle damage, and no significant differences existed between groups, consistent with published reports of untrained muscles (12, 21, 25). Muscle damage occurred in ~2% of fibers in baseline samples taken before training, similar to the 2–4% reported by Meltzer et al. (19) for muscles in young sedentary individuals.

The results of the present study are inconsistent with prior research, suggesting that older sedentary individuals exhibit higher levels of muscle damage in active skeletal muscle (vastus lateralis) than young sedentary individuals (19, 24, 26). Specifically, both Scelsi et al. (24) and Tomonaga (26) described myofibrillar degeneration with Z-band streaming in the muscles of older sedentary individuals. Tomonaga reported muscle damage in several skeletal muscles, both active and nonactive, whereas Scelsi et al. (24) reported damage in the vastus lateralis. Neither report quantified the total number of fibers assessed or the number of fibers that exhibited ultrastructural damage (24, 26). Further-
more, those investigations did not include young subjects as controls for comparison purposes (24, 26). The present investigation is the first that we are aware of to quantify the extent of muscle damage in both young and older sedentary men, either at baseline or in response to chronic HRST, and, furthermore, the first to show that no significant differences exist.

In the present study, the increase in the number of muscle fibers exhibiting muscle damage after 9 wk of HRST was significant in both training groups; however, no significant difference existed between groups. The increase in muscle damage exhibited in the young men in the present study is consistent with prior research. Staron et al. (25) reported ~9% of fibers exhibiting muscle damage in young strength-trained subjects, compared with untrained subjects, a value similar to the 7% reported for the young men in the present study. The increase in muscle damage to only 6% of assessed muscle fibers in the older men from the present study is a new finding, given the vigorous nature of the HRST stimulus that we applied. Although no direct comparison can be made, Manfredi et al. (17) used an acute eccentric exercise bout to assess differences in muscle damage in young and older men and found that >90% of the fibers obtained from the older men exhibited muscle damage compared with values of 5–50% of fibers reported for young men after a similar protocol (22). The results of the acute exercise bout indicated that the older men exhibited significantly higher levels of muscle damage compared with the young men, but the nature of that eccentric stimulus was different than that used in the present study and was designed to elicit skeletal muscle damage (17).

The strength increases demonstrated in the present study are similar to those reported in previous studies using lower body strength training in older men (3). However, the familiarization sessions associated with the present investigation before baseline strength testing may have diminished the magnitude of strength increases compared with other investigations, due to motor learning during the initial stages of training. The results of the present study also indicate a significant strength increase in the untrained leg in the older men after training. The untrained leg was positioned to prevent voluntary contraction. Thus the results suggest that the strength increase exhibited is likely due to cross-education of the untrained limb, a finding reported previously in both young and older individuals (3, 20).

In the present investigation, a number of fibers exhibited signs of damage likely due to the physical manipulations associated with tissue preparation. These fibers, often exhibiting hypercontraction (Fig. 1) and severe fraying or sarcolemmal disruption, were excluded from the analysis. Similar proportions of hypercontracted fibers in the untrained samples indicate that the observed structural alterations were not associated with the HRST program. Hypercontracted fibers are associated with the needle biopsy technique (13), and have been reported in previous studies (12, 17, 21). Whether the presence of hypercontracted fibers affected the interpretation of results in previous work is unclear. Hypercontracted fibers could be the result of either the biopsy/mincing procedures or the subsequent chemical fixation. Past research has indicated, however, that the use of gluteraldehyde before osmium fixation provides preservation of fine muscle structure, but there was no mention of fiber hypercontraction (8, 23). Damage associated with the needle biopsy technique has been described (25); however, the issue of hypercontracted fibers has not been evaluated to our knowledge. The structural alterations associated with muscle hypercontraction are different than those associated with strenuous exercise. As indicated in Fig. 1, the myofilament structure is maintained in hypercontraction, such that the M band can be distinguished and the Z disk, although possibly widened due to the crowding of myofilaments during hypercontraction, does not appear wavy or disrupted. Myofibrillar damage due to strenuous exercise is associated with distortion of myofilament structure and wavy or disrupted Z disks (Figs. 4–6). Because the damage associated with exercise is different from the structural alterations found in fiber hypercontraction, the elimination of these fibers from our analysis was warranted.

In the present study, a common feature of the after-training muscle fibers was the separation of myofibrils, possibly indicating the movement of fluid into the fiber (Fig. 7). A large proportion of fibers exhibited separa-

![Fig. 7. Electron micrograph of a skeletal muscle fiber exhibiting separation of myofibrils typical of after-training muscle fibers in both groups of subjects (magnification ×5,000).](http://jap.physiology.org/10.22033.2)
tion of myofibrils after the training in both groups, whereas this condition was rarely seen in before-training or untrained muscle samples. The intermediate filament desmin is associated with the Z bands of opposing myofibrils (9, 16). We speculate that the relatively uniform separation of myofibrils after training could be indicative of damage to these desmin filaments, although we have no data from the present study to support such a conclusion.

Although the increase in muscle damage after HRST, indicated in the present investigation, may suggest a disadvantage for older individuals with a reduced regenerative capacity, recent research indicates otherwise. Research assessing skeletal muscle nuclei and the regenerative muscle satellite cell indicates that the proliferative capacity of satellite cells is maintained even in old age, without the cellular signs of aging exhibited in other cell types (6). This, however, does not explain why older muscle seems to regenerate at a slower rate than does young muscle (2, 18). Research indicates that the host environment and, more specifically, neural factors play a major role in muscle tissue regenerative capacity (5). Despite this uncertainty, the results of the present study do not indicate that HRST is harmful to older muscle. In fact, past research suggests that the improved function after HRST in older individuals may increase independence in the performance of daily activities (7).

In summary, the results of the present investigation indicate that high-volume HRST leads to similar increases in muscle damage in both young and older men. Furthermore, the results of the present study are inconsistent with previous investigations, which have suggested that older sedentary men exhibit higher levels of baseline muscle damage compared with young sedentary men. The effects of HRST on nonactive muscles of young and older individuals remain to be determined. Future research should examine the effects of strength training on ultrastructural muscle damage in both older women and elderly individuals (~85 yr old).

We thank Tim Maugel, Director of the Laboratory for Biological Ultrastructure, and Dr. Dennis Goode for their expert technical advice and assistance. This work is contribution no. 85 from the Laboratory for Biological Ultrastructure at University of Maryland, College Park. We thank Dr. Colleen Farmer and the staff of the Wellness Research Laboratory for providing the exercise facilities. We also extend our appreciation to Mary E. Lott for help with training and to the subjects who participated in this investigation. This work was supported by the National Institutes of Health research contract 1AG-42148 (to B. F. Hurley and M. A. Rogers).

Address for reprint requests and other correspondence: M. A. Rogers, Department of Kinesiology, College of Health and Human Performance, University of Maryland, College Park, MD 20742 (E-mail: mr68@umail.umd.edu).

Received 6 October 1998; accepted in final form 10 February 1999.

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