The following is the abstract of the article discussed in the subsequent letter:

**Fiatarone Singh, MA, Ding W, Manfredi TJ, Solares GS, O’Neill EF, Clements KM, Ryan ND, Kehayias JJ, Fielding RA, and Evans WJ.** Insulin-like growth factor I in skeletal muscle after weight-lifting exercise in frail elders. Am J Physiol Endocrinol Metab 278: E754–E756, 2000.—To assess muscle remodeling and functional adaptation to exercise and diet interventions, 26 men and women aged 72–98 yr underwent a vastus lateralis biopsy before and after placebo control condition, and progressive resistance training, multinutrient supplementation, or both. Type II atrophy, Z band, and myofibril damage were present at baseline. Combined weight lifting and nutritional supplementation increased strength by 257±62% (P = 0.0001) and type I fiber area by 10.1±9.0% (P = 0.033), with a similar trend for type I fiber area (+12.8±22.2%). Exercise was associated with a 2.5-fold increase in neonatal myosin staining (P = 0.0009) and an increase of 491 ± 137% (P < 0.0001) in IGF-I staining. Ultrastructural damage increased by 141 ± 59% after exercise training (P = 0.034). Strength increases were largest in those with the greatest increases in myosin, IGF-I, damage, and caloric intake during the trial. Age-related sarcopenia appears largely confined to type II muscle fibers. Frail elders respond robustly to resistance training with musculoskeletal remodeling, and significant increases in muscle area are possible with resistance training in combination with adequate energy intakes.

**Interpretation of Muscle Damage From Fixed Tissue Obtained by Needle Biopsy**

To the Editor: Recently, Fiatarone Singh et al. (5) reported data outlining muscle damage in frail elders, free of myopathy, before and after strength training. The authors reported that the levels of muscle damage exhibited by their elderly subjects before training were higher than levels exhibited in young individuals, although muscle samples from young subjects were not analyzed for comparison (5). As an example of ultrastructural muscle damage, the authors presented a micrograph (Fig. 3A in Ref. 5) exhibiting hypercontraction and “wide sarcoplasmic spaces.” We contend that the structural disruption demonstrated in that micrograph is an artifact of the needle biopsy procedure, rather than age-associated myofibrillar degeneration.

Muscle damage analysis in many cases relies on the use of needle biopsy (1) and postbiopsy mincing procedures, after which the tissue sample is fixed using a glutaraldehyde buffer and osmium tetroxide (6, 7, 10), all of which may potentially affect muscle structure and thus interpretation of muscle damage. Although the methods outlined by Fiatarone Singh et al. (5) follow these standards, their interpretation of the resulting micrographs is uncertain. As indicated by Fig. 3A (5), the micrograph chosen as an example of baseline muscle damage clearly demonstrates intact M lines, shrunken and separated myofibrils, and hypercontraction, rather than Z-disk streaming or myofibrillar disruption, all evidence of alterations due to the biopsy procedure rather than to degeneration intrinsic to the muscle (2). In fact, the micrograph in Fig. 3A (5) is similar to a micrograph presented by our group as an example of a muscle fiber likely disrupted by the tissue biopsy or fixation process (Fig. 1 in Ref. 10). We similarly observed intact M lines with hypercontracted myofibrils, leading to an apparent widening of the Z disk; however, we found similar proportions of hypercontracted fibers in both young (20–30 yr) and older (65–75 yr) men in baseline and strength-trained conditions, in addition to control leg muscle samples, indicating that the observed structural alterations were not associated with aging or the strength training intervention, but rather with the biopsy procedure (10).

Hypercontracted fibers have been observed in several exceptional settings (e.g., in dystrophic muscle and after lidocaine administration) (3, 9), likely the result of high Ca²⁺ concentrations (4). Although limited evidence exists that extreme eccentric muscle actions may result in hypercontraction in normal muscle, the regeneration associated with such damage includes infiltration of mononuclear inflammatory cells within 24 h of the stimulus (7). Thus the presence of noninfilitrated hypercontracted fibers after the biopsy procedure in nondiseased muscle in the work of Fiatarone Singh et al. (5) indicates that mechanical factors are likely involved. Hypercontracted fibers are associated with the needle biopsy/mincing technique (2,8). Because the use of glutaraldehyde buffer before osmium tetroxide fixation provides preservation of fine muscle fiber structure (6, 11), we contend that the structural disruption observed in hypercontracted fibers is a consequence of the needle biopsy and/or postbiopsy mincing procedures and cannot be clearly interpreted as age-associated muscle damage.

Whereas the second micrograph (Fig. 3B) in the work of Fiatarone Singh et al. (5) provides evidence of muscle degeneration after the strength training program, the interpretation of the level of muscle damage in both baseline and trained muscle samples is questionable on the basis of evidence noted above. Likewise, the comparison of muscle damage levels in the elderly subjects with young individuals in a separate investigation is suspect. Although the qualitative change in muscle damage with strength training is not contested, we suggest that the quantification of muscle damage is skewed by the inclusion of hypercontracted fibers. Because muscle...
The letter by Roth and Rogers suggests that the use of the needle biopsy and postbiopsy mincing procedures before tissue fixation may introduce muscle fiber hypercontraction and artifact, which can be interpreted as muscle damage. They also imply that Fiatarone Singh et al. (Fig. 3A, Ref. 2) may have misinterpreted a high incidence of Z band damage and wide sarcoplasmic spaces as ultrastructural features of muscle biopsies taken from frail elderly men and women. Roth and Rogers contend that these features reported by us (2) are more likely due to artifact. They attempt to support this statement with a similar micrograph (Fig. 1 of Ref. 12) of muscle fibers taken with a needle biopsy by their group, and they contend that this figure shows evidence of damage due to the biopsy procedure and tissue mincing. It is clear that two micrographs share evidence of wider than normal Z bands.

Our laboratory has used the technique described in this paper for more than 15 years to examine the phenomenon of exercise-induced muscle damage (2–5, 8, 11, 13, 14). We have quantified sarcomere (9) damage in young and older adults after eccentric exercise and have never observed baseline muscle damage similar to that seen in the very old subjects examined in our recent paper. These earlier studies have demonstrated that 4.8% of the Z bands were damaged in healthy young sedentary men, aged 22–29 yr at baseline (4), and that 4–28% were damaged in healthy young men, aged 18–30 yr (6), also at baseline. When damaged Z bands were presented as a percentage volume density of muscle, we saw that <0.5% of the muscle had damaged Z bands in young (20–29 yr) and older (60–75 yr) men (7). Although we have quantified muscle damage by use of different criteria, young subjects in our studies have consistently demonstrated minimal muscle damage at baseline compared with subjects described by Roth et al. (12). We (9) have reported no evidence of preexisting focal damage in subjects aged 59–63 yr in a study which employed a similar method to that of Roth et al. for quantification of sarcomere damage (we examined more fibers/biopsy). This study employed a similar technique that has also previously been used in describing muscle damage (10).

We therefore disagree with Roth and Rogers that the 20–21% volume density of damaged Z bands in our very old subjects was due to artifact and tissue mincing. Few if any studies to date have provided more extensive and quantitative ultrastructural examination of muscle from the oldest of the old than that reported in our study (2). There is reason to believe that the existence of damage in very old individuals is a consequence of mechanisms that have been previously described (1). Our earlier studies provide rigorous evidence that this baseline muscle damage is a feature of the extremely old individuals recruited for this investigation and not an artifact of sample preparation. We do not disagree with Roth and Rogers that the muscle damage observed in their study may have been related to their needle biopsy procedure and/or tissue handling.

It is also important to point out that our study (2) was a randomized trial with a sedentary control group. There was no change in the amount of Z band or myofibrillar damage in the control subjects at the 10-wk time point compared with baseline, whereas the exercising subjects demonstrated an average 141% increase in Z band damage and a 589% increase in myofibrillar damage (Table 4 of Ref. 2). Artifact cannot explain such group effects, given the identical biopsy procedures used in both groups at each time point. These findings of increased damage were highly correlated to the changes in strength in the exercised muscles, indicating that the exercise-induced muscle damage may have metabolic relevance. We believe that the suggestion that our results are “suspect” is incorrect.
REFERENCES


William J. Evans  
Nutrition, Metabolism, and Exercise Laboratory  
University of Arkansas for Medical Sciences  
Maria Fiatarone Singh  
School of Exercise and Sport Science, Cumberland Campus  
University of Sydney, Australia  
Thomas Manfredi  
University of Rhode Island  
Roger Fielding  
Boston University