Discussion. From the results it appears that although mean RR and HR showed no significant changes HRv and RRv are sensitive, with HRv being more responsive and also showing a between-conditions effect. Seemingly, the high visual load condition did potentiate the effects of TSD, indicating that experimental conditions of TSD do play a major role. Correlating HRv and RRv scores for each subject over both TSD conditions produced an overall average product moment correlation of 0.78. This 61% common variance indicated that the 2 variables are significantly correlated. Because respiratory sinus arrhythmia (RSA) is a non-linear, respiratory depth and frequency dependent phenomenon[19,20] varying in phase and magnitude, and as RR was allowed to vary, a more detailed partialling out of a RSA component from the HRv data was not possible.

Circadian rhythms in the data were not statistically evident. However, as there were only 4 measures per day, with pooling of data, and a possible circadian rhythm disruption owing to TSD, such an effect might not be obvious.

More sophisticated[15,16] HRv and RRv indices could have been employed but the present method, although simple, was apaprently effective. From observing both subjects and raw data it appears that the RRv increases were often due to increases in yawning and sighing, etc.

The exact role of CNS fatigue and any CNS impairment to HR and RR control cannot be established here, however these subjects did show increasing amounts of behavioural fatigue, especially during the high load condition. These effects are detailed elsewhere[21].

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Changes in Z-disc width of vertebrate skeletal muscle following tenotomy[1]

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Summary. Results of unilateral Achilles tenotomy on male rats, after 2–6 weeks, showed conclusively that the Z-lines of tenotomized muscles are significantly wider than those of control, nontenotomized muscles.

The Z-line of vertebrate skeletal muscle is known to become morphologically altered (rod or nemaline bodies, streams, etc.) in a variety of pathological conditions[3,4] as well as under conditions of hypertrophy[5,6] and tenotomy[7,8]. Figure 1 shows the typical streams and rod bodies following tenotomy. Also Fujisawa[9] found streams in otherwise normal aged rats. He noted that the Z-lines in those affected fibres appear wider (up to 120 nm) than in non-streaming fibres (up to 92 nm). Although the exact function and chemical nature of the Z-discs are not known, it has been suggested that the structural proliferation observed is the first step in the normal development of new sarcomeres[5,10,11].

In the specific case of tenotomy, if one assumes that the stimulus, perhaps a sudden release of resting muscle tension, induces a 'proliferative' response in all the Z-lines of the affected muscle, then this should be detectable by measurement of Z-line width in areas of the muscle which do not exhibit the more obvious and familiar Z-line abnormalities. The following study, therefore, was designed to answer the simple question: Are the Z-lines of tenotomized muscle, apart from those showing rod formation or streaming, thicker as compared to the Z-lines of a comparable, but non-tenotomized muscle?

Materials and methods. Following the method described by Shafiq et al.[7], Achilles tenotomy, including removal of a 2–3 mm segment of tendon, was performed on 1 leg of 150–300 g male albino rats, with the unoperated egl serving as the control. Following a period of 2–6 weeks, the animals were sacrificed by decapitation and the soleus muscles excised and placed in ice cold buffer (0.1 M KCl, 1 mM $MgCl_2$, 5 mM EGTA, 5 mM sodium pyrophosphate, pH 6.8). Under the dissecting microscope, fibres were dissected free, tied to 3 cm fragments of wooden applicator sticks at approximately rest length, and then placed at 4 °C in 4% glutaraldehyde in buffer (7.5×10^{-2} M KCl, 7.5×10^{-4} M $MgCl_2$, 7.5×10^{-3} M Na_2HPO_4, 7.5×10^{-3} M KH_2PO_4, pH 7.0). The samples were then post-fixed in 1% OsO_4 for 1 h, dehydrated in a graded series of ethanol and embedded in DDSA/araldite. Longitudinal thin sections were cut at 60–70 nm on a Porter-Blum MT 2 microtome, and mounted on 200 mesh copper grids. The sections were then stained with 1% PTA, 10% uranyl acetate, and Reynold's lead citrate and examined with a Philips 300 electron microscope. Electron micrographs were taken at the same magnification of both the control and experimental soleus muscles, care being taken to avoid areas in the tenotomized muscle that abounded with the rod shaped and streaming Z-structures.

Direct measurement were taken (independently, by each author) from 8×10 inch enlargements of the various plates using a metric ruler for sarcomere and A-band length, and a desk-top magnifier containing a reticle with

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Effect of tenotomy on soleus muscle Z- and M-line widths, A-band width, and sarcomere length

Paired muscles*	Duration of tenotomy, weeks	Sarcomere length, μm**	A-band length, μm**	M-line width, nm**	Z-line width, nm**
C3S	6	1.83	1.31	69.03	101.52
E3S	6	2.09	1.39	73.63	132.26
C4S	6	2.41	1.55	84.21	111.77
E4S	6	2.30	1.46	96.35	144.59
C10S	2	2.08	1.35	85.47	112.82
E10S	2	2.82	1.32	85.47	120.51
C12S	4.5	2.18	1.28	79.15	93.76
E12S	4.5	3.11	1.39	75.91	116.33
C27S	3	2.68	1.32	71.83	110.86
E27S	3	2.62	1.36	69.57	127.26
C29S	3	2.13	1.31	74.83	121.72
E29S	3	2.46	1.29	82.24	125.74
Mean difference		0.35	0.02	3.11	19.04
SD of mean difference		0.42	0.08	6.02	11.84
p value, paired comp.		>0.05	>0.05	> 0.05	< 0.02

*Specimens labelled 'C', represent control muscle; 'E', contralateral tenotomized muscle. **Values represent means of measurement within each muscle.

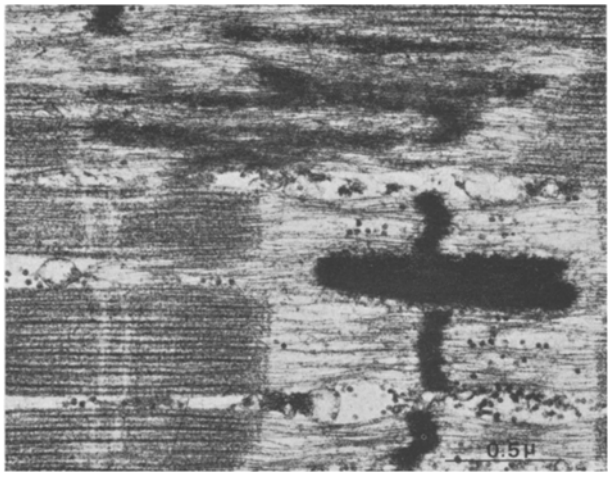

Fig. 1. Longitudinal section of tenotomized rat soleus muscle, showing rod body and adjacent 'streaming' Z-line. ×48,000.

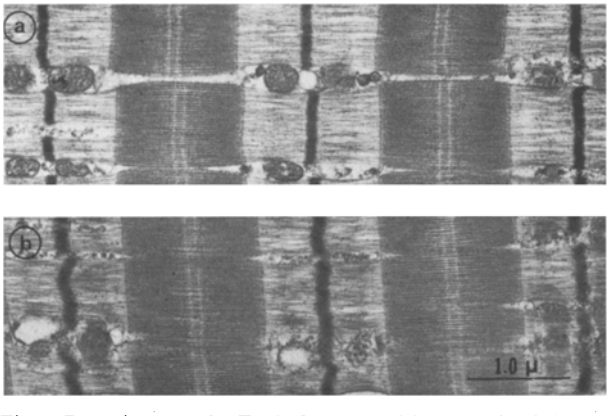

Fig. 2. Rat soleus muscle: Typical areas used for analysis. a) Control (27S). b) Experimental (27S). In this animal, the average Z-line width was 15% greater in the tenotomized muscle than in the contralateral control. ×19,720.

0.1 mm graduations for Z- and M-line widths. As Z-lines tend to be very irregular across the width of the myofibril, measurements were taken at multiple intervals along each Z-line in the micrograph.

Results. Figure 2a and b are electron micrographs of the soleus muscles from a rat, showing typical areas used for analysis. The portion of the tenotomized muscle (figure 2b) shown has no obvious large Z-line alterations present, yet measurements proved the tenotomized Z-lines to be significantly wider than the control muscle by approximately 15%. The table presents the results of our analysis from 6 tenotomized animals. A total of 520 'control' Z-lines were measured, and compared to a total of 505 'tenotomized' Z-lines. A paired comparison test showed that the Z-lines were significantly wider ($p < 0.02$) by about 19 nm than the Z-lines of the non-tenotomized muscles. Between the 2 groups of muscles, there was no significant difference in the M-line or A-band width, or in sarcomere length.

Discussion. It has long been recognized that the Z-lines of fast (white, type 2) fibres are significantly narrower (by approximately half) than those of slow (red, type 1) fibres. The soleus muscle used in this study is considered predominantly type 1. Gauthier and Dunn[12] using rat semitendinosus muscle determined fast fibres to have Z-discs 40 nm in diameter compared to 80 nm in slow fibres. Shafiq et al.[13] using chicken sartorius found fast fibres with Z-discs 63 nm thick as opposed to 80 nm for slow fibres. Then, in 1974, Gauthier[14], working only with soleus muscles from rats and guinea pigs found wide Z-lines in both the slow and intermediate fibres (100 nm in rat, 120 nm in guinea pig) and suggested that soleus fibres represent a separate category of fibres that resemble red/slow fibres but which possess an unusually wide Z-line. This idea receives support from the work of Eisenberg and Kuda[15] who noted a range of Z-width in guinea pig vastus lateralis red fibres of 60–130 nm (mean: 88.1) compared to guinea pig soleus fibres of 100–190 nm (mean: 144.5). Compared to Gauthier's 1974 data, our control rat soleus muscles are above her mean figure of 100 by about 9%, but considering differences in technique and internal variability of measurement of this sort, the difference is probably not significant.

We did attempt to restrict our measurements to the type 1 fibres on the basis of M-line width and mitochondria content[16,17], but typing fibres based on electron microscopy is difficult at best[18]. Measurements of sarcomere length and A-band width were made to determine whether tenotomy affected those parameters in addition to the alterations noted in Z-lines: We found no apparent differences.

Since our results on the Z-line width are positive, we consider then that tenotomy causes a generalized reaction in all the Z-lines of the affected muscle. Perhaps the various forms seen in this condition represent different stages of the same underlying process.

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