A physiological level of clenbuterol does not prevent atrophy or loss of force in skeletal muscle of old rats

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Chen, Kuangjen D., and Stephen E. Alway. A physiological level of clenbuterol does not prevent atrophy or loss of force in skeletal muscle of old rats. J Appl Physiol 89: 606–612, 2000.—Supraphysiological levels of clenbuterol (CL) reduce muscle degradation in both young and old animals; however, these pharmacological levels induce side effects that are unacceptable in the elderly. In this study, we tested the hypothesis that a “physiological” dose of CL (10 µg·kg⁻¹·day⁻¹) would attenuate the loss of in situ isometric force and mass in muscles of senescent rats during hindlimb suspension (HS). Adult (3 mo) and senescent (38 mo) Fischer 344 × Brown Norway rats received CL or a placebo during 21 days of normal-weight-bearing or HS conditions (8 rats/age group). HS reduced soleus muscle weight-to-body weight ratio by 31%, muscle cross-sectional area by 37%, and maximal isometric tetanic force (Po) by 76% in senescent rats. CL attenuated the loss of Po and muscle weight by 17 and 8%, respectively, in the soleus of senescent rats relative to HS + placebo conditions, but it did not improve muscle weight normalized for body weight. CL did not reduce the decrease in soleus Po or mass after HS in adult rats. CL failed to reduce the loss of plantaris weight (−20%) and Po (−46%) in senescent rats after HS. Our data support the conclusion that physiological levels of CL do not improve fast muscle atrophy and only modestly reduce slow muscle atrophy, and, therefore, it is largely an ineffective countermeasure for preventing muscle wasting from HS in senescent rats.

aging; disuse; β-agonist; muscle strength; isometric contraction; hypoknesia; myosin heavy chain; sarcopenia

β₂-ADRENERGIC AGONISTS such as clenbuterol (CL), salbutamol, and cimaterol have been used as a countermeasure for the treatment of muscle-wasting disorders because these compounds stimulate muscle growth and protein accumulation in muscles from young adult animals (1, 6, 9, 10, 12, 25). CL has been shown to reduce muscle atrophy under conditions of hindlimb suspension (HS) (5), hyperthyroidism (11), surgery (9), and denervation (1, 6, 25). However, studies showing anabolic changes have typically used supraphysiological doses of CL from ~0.2 to 2.0 mg·kg⁻¹·day⁻¹ (10, 11, 12, 18), and these levels exceed the equivalent estimated safe dose in humans (26).

Aging-associated atrophy (sarcopenia) reduces the initial muscle mass “reserve” and strength of elderly humans and rodents (3, 13). This is a potentially important problem because aging increases the sensitivity of muscle fibers to inactivity-induced atrophy (2, 15, 33, 37). Thus it appears possible that superimposing disuse or immobility with sarcopenia (e.g., recovery from surgery or a fall) may further exacerbate the loss of muscle mass and strength.

To our knowledge, no study has examined whether administration of CL within safe physiological levels is an effective countermeasure for non-weight-bearing-related atrophy and loss of contractile function in muscles from aging animals. In this study, we chose to use HS as a model of non-weight-bearing disuse in senescent rats because it invokes significant muscle loss in rodents (16, 19, 30, 31, 36). Therefore, we tested the hypothesis that treatment with a low (i.e., “physiological”) level of CL would attenuate both the loss of muscle protein and in situ contractile force in hindlimb muscles of senescent rats during non-weight-bearing conditions of HS. Second, our pilot data indicated that HS induced atrophy of the soleus (Sol) in both young and senescent rats and in the plantaris (Pl) of senescent rats. Because we did not expect to find significant atrophy in the Pl of adult rats after HS, we did not anticipate finding any measurable effect of physiological levels of CL in the Pl of young adult rats. However, we rationalized that, because aging is associated with sarcopenia and especially type II fiber atrophy (3), CL would have a greater effect on the Pl fibers from senescent rats, if β₂-receptors were not significantly downregulated in these atrophied fast fibers, because this would improve their receptor density compared with the larger fibers of young animals. Therefore, the second hypothesis we tested was that CL would preserve muscle mass and function in the Pl of senescent rats.

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HINDLIMB UNWEIGHTING AND CLENBUTEROL IN AGED RATS

Table 1. Soleus muscle characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control (Young adult)</th>
<th>Control (Senescent)</th>
<th>HS+Placebo (Young adult)</th>
<th>HS+Placebo (Senescent)</th>
<th>HS+CL (Young adult)</th>
<th>HS+CL (Senescent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle wet wt, mg</td>
<td>172 ± 10</td>
<td>158 ± 12</td>
<td>124 ± 8†</td>
<td>109 ± 5§</td>
<td>111 ± 8§</td>
<td>122 ± 5†</td>
</tr>
<tr>
<td>Muscle wt/body wt, mg/g</td>
<td>0.61 ± 0.03</td>
<td>0.34 ± 0.03</td>
<td>0.42 ± 0.02‡</td>
<td>0.28 ± 0.04*</td>
<td>0.37 ± 0.03‡</td>
<td>0.29 ± 0.03*</td>
</tr>
<tr>
<td>Muscle CSA, mm²</td>
<td>6.2 ± 0.5</td>
<td>4.6 ± 0.37</td>
<td>4.5 ± 0.3‡</td>
<td>2.9 ± 0.2*</td>
<td>4.2 ± 0.3‡</td>
<td>3.6 ± 0.2†‡</td>
</tr>
<tr>
<td>Total protein, mg/g</td>
<td>69.1 ± 4.1</td>
<td>67.2 ± 4.7‡</td>
<td>89.4 ± 5.2‡</td>
<td>60.4 ± 3.1*‡</td>
<td>83.9 ± 3.9†</td>
<td>62.9 ± 5.2‡</td>
</tr>
<tr>
<td>Myofibril protein, mg/g</td>
<td>61.9 ± 7.8</td>
<td>39.0 ± 3.7*</td>
<td>27.5 ± 3.3‡</td>
<td>21.2 ± 2.6*‡</td>
<td>28.6 ± 4.3§</td>
<td>26.8 ± 2.8*‡</td>
</tr>
<tr>
<td>Myofibril protein/RNA content</td>
<td>1.37 ± 0.10</td>
<td>1.14 ± 0.08*</td>
<td>1.13 ± 0.07‡</td>
<td>0.93 ± 0.06*‡</td>
<td>0.92 ± 0.08*</td>
<td>0.97 ± 0.05*</td>
</tr>
<tr>
<td>RNA content, mg/g</td>
<td>47.4 ± 4.1</td>
<td>34.6 ± 3.6*</td>
<td>26.5 ± 2.1§</td>
<td>23.7 ± 3.5§</td>
<td>32.4 ± 4.4§</td>
<td>27.3 ± 2.6‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. HS+placebo, group treated with hindlimb suspension and placebo; HS+CL, group treated with HS and clenbuterol; CSA, cross-sectional area. *Young adult vs. senescent rat in the same experimental condition, P < 0.05. †HS+placebo vs. HS+CL conditions in age-matched groups, P < 0.05. ‡Significantly different from age-matched control muscle, P < 0.05.

METHODS

Animal care and HS. All procedures followed the guidelines of the National Institutes of Health, and they were approved by the Institutional Animal Care and Use Committee. Twenty-four young adult (3 mo) and twenty-four senescent (35 mo) male Fischer 344 × Brown Norway rats were obtained from the National Institute on Aging barrier-raised colony that is housed at Harlan Animal Colonies (Indianapolis, IN). The animals were housed at 20–22°C in barrier-controlled conditions under a 12:12-h light-dark cycle. They were provided rat chow and water ad libitum.

The rats in each age group were randomly assigned to a HS (n = 16) or a control group (n = 8). The HS animals were suspended by orthopedic tape and a metal cable harness that was applied to the proximal two-thirds of the tail. This distributed the load along the length of the tail, thereby avoiding excessive tension on a small area. The exposed tip of the tail remained pink, indicating that HS did not interfere with blood flow to the tail. HS suspension was maintained for 21 days. The height of suspension was adjusted to prevent the hindlimbs from touching any supportive surface. The forelimbs maintained contact with a grid floor, which allowed the animals to move and to obtain food and water freely. Control rats maintained normal mobility and they moved unconstrained around their cages.

CL treatment. Rats in the HS group were implanted with a pellet (Innovative, Sarasota, FL) that released CL at a rate of 0.01 mg · kg body wt⁻¹ · 24 h⁻¹ (HS+CL group) or a pellet that released a placebo vehicle (HS+placebo group). Control experiments were conducted in rats of similar body weights by the manufacturer of the pellets to verify the constant-release nature of the compounds. The pellets were implanted subcutaneously in the interscapular area by using a general anesthesia (xylazine hydrochloride and ketamine hydrochloride ip). The wound was closed with suture and observed throughout the course of the study to ensure that infection did not develop.

In situ isometric measures. After 21 days, the animals were anesthetized with ketamine hydrochloride (9 mg/100 g body wt) and xylazine hydrochloride (1 mg/100 g body wt). In situ isometric contractile measures were made from the Sol and PL muscles from the right leg. The limbs were fixed, and the distal tendons of each muscle were sectioned just proximal to the calcaneus and attached by epoxy resin to a hook on the
lever arm of a FORT-250 (Sol measures) or a FORT-1000 (Pl measures) force transducer (World Precision Instruments, Sarasota, FL). The force transducers were mounted on two-dimensional micromanipulators (Newport, Irvine, CA) for adjustment of optimal muscle length ($L_o$). The muscle length that produced the greatest twitch force was taken as $L_o$. Muscles were equilibrated in a rat Ringer bath for at least 30 min after surgery. The output signal from the force transducers was amplified and recorded on a personal computer for off-line analysis. The Sol and the Pl muscles were maximally activated at $L_o$ by simulating the tibial nerve with a direct-current square-wave pulse. A power amplifier was used to boost the current output of a Grass SD9 stimulator. The pulse duration was 0.2 ms with a voltage twice that necessary to elicit a maximal twitch. Peak isometric tetanic tension ($P_o$) was determined at a stimulation frequency of 100 Hz. A minimum of two tetanic contractions were made with the greatest of the measurements taken as $P_o$. A recovery interval of 5 min was taken between each contraction.

**Muscle CSA.** Physiological muscle cross-sectional area (CSA) is proportional to muscle force; therefore, it was important to know whether changes in CSA would account for the changes in isometric force. After contractile measures, the muscles of both legs were dissected, cleaned of excess connective tissue, weighed, frozen at $L_o$ in liquid nitrogen, and stored at $-80^\circ$C. CSA was estimated from frozen 10-μm-thick tissue cross sections that were cut from the midbelly of Sol and Pl muscles of the left leg. The sections were stained with hematoxylin and eosin, mounted in glycerol, and viewed under a light microscope (Zeiss, Orthoplan) at an objective magnification of ×10. The signal from a video camera mounted to the microscope was sent to a video frame grabber and a personal computer. Muscle CSA was determined by planimetry from a commercial morphometric software package (Biometrics). The CSA for each muscle was calculated from the average of three planimetry records. These measurements were obtained with the investigators blinded to the experimental group.

**Protein and myosin heavy chain analyses.** Protein and myosin heavy chain (MHC) analyses were made to determine whether CL treatment would attenuate the loss of force, myofibril protein, and fast myosin-to-slow myosin shift that have been well documented with HS (16, 19, 30, 31, 33, 36, 37). The left Sol and Pl muscles were used for analysis of protein and RNA content. Myofibrils were isolated from whole muscle homogenates according to the methods previously described (16). Briefly, muscles were homogenized in a cold buffer that contained (in mM) 250 sucrose and 20 imidazole, pH 7.0. The pellet was washed in a 0.5% Triton X-100 buffer containing (in mM) 175 KCL, 5 EDTA, and 20 imidazole, pH 7.0, and was centrifuged at 1,200 g. The pellet was homogenized in a buffer containing (in mM) 150 KCL and 20 imidazole, pH 7.0, and centrifuged at 1,200 g, and the myofibril pellet was resuspended in this same buffer at 3 mg/ml. The protein concentration of the myofibril suspension was assayed spectrophotometrically at 562 nm (Dynex MRX microplate reader) in triplicate by a commercial bicinchoninic acid method as recommended by the manufacturer (Pierce, Rockford, IL) with bovine serum albumin used as a standard.

Myofibril proteins were subjected to standard 8% SDS-PAGE in a Bio-Rad Mini- Protean II Dual Slab Cell (Hercules, CA) for 24 h at 4°C at 120 V as described by Talmadge and Roy (35). The stacking gels were composed of 30% glycerol, 4% N,N’-methylene-bis-acrylamide (bis), 70 mM Tris (pH 6.7), 4 mM EDTA, and 0.4% SDS. The separating gels were composed of 33% glycerol, 8% acrylamide/bis (50:1), 0.2 M Tris (pH 8.8), 0.1 M glycine, and 0.4% SDS. Polymerization

![Fig. 2. Effects of 21 days of CL and HS on relative myosin heavy chain (MHC) protein isoform content in soleus muscles from young adult and senescent rats. A: type I MHC. B: type IIa MHC. C: type IIx MHC. Values are means ± SE from 8 rats that were averaged for each experimental group. Individual data points were obtained from averaging quantified data obtained from 2 separate optical scans of each lane of each SDS gel. Expression of each MHC isoform is expressed relative to total MHC isoform pool. a Young adult group significantly different from senescent rat group in the same experimental condition, P < 0.05. * Significantly different from age-matched control group, P < 0.05.](http://jap.physiology.org/content/images/214x214)
was initiated with 0.1% ammonium persulfate and 0.05% EDTA. Each gel lane was loaded with 0.5 μg of myofibrillar protein, and the MHC bands were visualized with silver (Bio-Rad). Identifications of the MHC bands were compared with published records under the same conditions (35) and also by Western blotting (data not shown). Each lane was scanned two times with an optical scanner, and each scan was quantified by using Bio Image (Genomic Solutions). The data from both scans were averaged for each lane. Typically, the data from two consecutive scans were nearly identical (range of 0–0.3% difference between consecutive scans and analyses). Preliminary experiments indicated that the optical signal remained linear when 0.3–0.7 μg of myofibrillar protein was loaded per lane of the SDS gel. The relative MHC expression was determined by expressing the integrated signal for a given MHC band as a percentage of the total signal for all MHC bands in that gel lane.

RNA isolation. Total RNA is mainly ribosomal; therefore, so the ratio of RNA to protein was used as a crude indicator of the muscle’s potential for transcription and/or translation would be improved by CL (with a greater ratio indicating an increased potential for protein synthesis). Total RNA was isolated from frozen muscle samples (~100 mg) from the left leg, which were homogenized in 1 ml of Tri-Reagent (Molecular Research Center, Cincinnati, OH) in duplicate and accepted if the 260-to-280 nm ratio was >1.6. RNA was quantified by 260 nm and then stored at −80°C. Typically, repeat measures for RNA varied no more than 0.01 optical density unit at 260 nm, which is within the error of sensitivity of the spectrophotometer.

Statistical analysis. Data are presented as means ± SE. Physical characteristics, contractile properties, myosin isoforms, protein content, and RNA content were analyzed by a two-way analysis of variance (age × condition). Tukey’s test was employed for post hoc analyses. P < 0.05 was selected to indicate statistical significance.

RESULTS

Body weight. Body weight was greater in senescent rats (control, 467 ± 10 g) compared with young adult rats (control, 298 ± 9 g). HS did not significantly affect body weight in young adult rats (296 ± 11 g), whereas HS significantly decreased body weight in senescent rats (398 ± 11 g). CL did not alter body weight in young adult rats (297 ± 10 g), and it did not significantly improve the HS-associated loss of body weight in senescent rats (423 ± 9 g).

Sol muscle characteristics. CL did not reduce the HS-associated loss of muscle force in the Sol of adult rats (Table 1, Fig. 1). Consistent with this finding, CL failed to alter the relative decline in CSA, myofibrillar protein, or total RNA in the Sol of the young adult rats after HS (Table 1, Fig. 1). In senescent rats, CL modestly attenuated the HS-induced decrease in Sol Po by 17 ± 1% (Fig. 1) relative to the losses incurred under HS + placebo conditions; however, the loss of relative tension as indicated by force/CSA was not diminished by CL (Fig. 1). The inactivity-induced loss of myofibrillar protein concentration and muscle CSA was attenuated by 15 ± 1% in senescent HS + CL rats compared with HS senescent rats (Table 1). MHCs did not differ in CL and placebo conditions after HS (Fig. 2).

Pl muscle characteristics. Pl wet weight, protein concentration, total RNA, and Po were similar in control, HS, and HS + CL groups (Table 2, Fig. 1). In contrast, HS reduced Po by 46 ± 3% and decreased the muscle weight-to-body weight ratio and the estimated Pl muscle CSA by 6 ± 1 and 20 ± 2%, respectively, in senescent rats. CL did not significantly improve relative tension in the Pl of senescent rats after HS (Fig. 1). Neither total protein nor total RNA was significantly reduced by HS in Pl muscles of either age group relative to control muscles. Myofibrillar protein was reduced

### Table 2. Plantaris muscle characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HS + Placebo</th>
<th>HS + CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plantaris wet wt, mg</td>
<td>366 ± 14.1</td>
<td>333.4 ± 20.4</td>
<td>313.7 ± 8.9</td>
</tr>
<tr>
<td>Muscle wt/body wt, mg/g</td>
<td>1.23 ± 0.04</td>
<td>1.16 ± 5.2</td>
<td>1.03 ± 4.7</td>
</tr>
<tr>
<td>Plantaris CSA, mm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>8.72 ± 0.29</td>
<td>7.45 ± 0.40</td>
<td>7.47 ± 0.21</td>
</tr>
<tr>
<td>Total protein, mg/g muscle</td>
<td>109.2 ± 4.6</td>
<td>108.1 ± 3.2</td>
<td>113.2 ± 4.9</td>
</tr>
<tr>
<td>Myofibril protein, mg/g muscle</td>
<td>42.3 ± 7.4</td>
<td>32.3 ± 3.7</td>
<td>65.1 ± 3.5</td>
</tr>
<tr>
<td>Myofibril protein/RNA content</td>
<td>0.79 ± 0.08</td>
<td>0.56 ± 0.04†</td>
<td>0.57 ± 0.03</td>
</tr>
<tr>
<td>RNA content, mg/g muscle</td>
<td>0.68 ± 0.03</td>
<td>0.62 ± 0.06</td>
<td>0.67 ± 0.05</td>
</tr>
<tr>
<td>Myofibril protein/RNA content</td>
<td>117.8 ± 9.9</td>
<td>93.4 ± 9.1†</td>
<td>86.6 ± 4.8†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. *Young adult vs. senescent rat in the same experimental condition, P < 0.05. †Significantly different from age-matched control muscle, P < 0.05.

![Fig. 3. Representative SDS gels for plantaris muscle after HS in young adult and senescent rats. Protein (0.5 μg) was loaded on each lane, and gels were stained with silver. Positions for bands corresponding to type IIa, IIx, IIb, and I MHCs are indicated in each gel.](http://jap.physiology.org/Downloaded from HINDLIMB UNWEIGHTING AND CLENBUTEROL IN AGED RATS by 10.220.33.5 on June 8, 2017)
by nonweight bearing in PL muscles of both young adult rats (26 ± 2%) and senescent rats (24 ± 2%), but CL did not reduce this decrease (Table 2). The expression of type IIa MHC in the PL from senescent rats was 8 ± 0.7% greater in the HS+CL group compared with the HS+placebo group, whereas type IIx MHC was 8 ± 2% lower in the HS+CL group compared with the HS+placebo group (Figs. 3 and 4).

**DISCUSSION**

In this study we show for the first time that a low dose of CL (10 μg · g⁻¹ · day⁻¹) that would be considered within physiological ranges can only modestly attenuate HS-induced loss of Po in the Sol of senescent rats. Supraphysiological levels of CL affect skeletal muscle growth by decreasing the rate of protein degradation (7, 29). Three pieces of evidence suggest that this is also the mechanism by which CL modestly reduces Po by delaying muscle atrophy during HS in senescent rats. First, the HS+CL senescent rats had smaller decreases in muscle CSA (15%) and protein concentration (12%), and these were of the same magnitude as the reduction in the loss of Po (17%) compared with the HS+placebo senescent rats (Table 1). Second, although Sol muscle weight was greater in HS+CL vs. HS+placebo senescent rats, this positive effect of CL was negated when Sol wet weight was normalized for body weight (Table 1). Third, the intrinsic properties of muscle force after HS were unaffected by CL because Po normalized to muscle CSA was similar in HS+placebo and HS+CL senescent rats. Furthermore, the subtle preservation of type IIa MHC with CL administration (Fig. 2) in the Sol of senescent rats had no effect on intrinsic force production. Although we cannot rule out the possibility that a small part of the improvement in overall Sol force may have been due to a potentiation of excitation-contraction coupling by CL (8), this seems unlikely because P_o/CSA was unchanged by CL. Together these data suggest that the mass effect of slowing the total loss of muscle mass (and especially CSA) by CL resulted in a slight reduction in the degree of Sol force reduction in senescent rats.

We hypothesize that there are at least two possible routes whereby CL delayed muscle protein loss during HS in Sol of old rats. The first possibility is that the β₂-receptor density may be altered in the Sol of old senescent rats. Sarcopenia is thought to be due in part to motoneuronal death, because some fibers in mammals become denervated with aging (4, 21). If denervation resulted in an upregulation of the β₂-receptors of the slow Sol fibers (25) of senescent rats, these denervated fibers would bind more total CL under conditions of low physiological dose of CL than would the fully innervated Sol muscle of the young adult rat. This would explain the slowing of muscle wasting in the muscles from senescent animals compared with young rats. Consistent with this idea, Maltin et al. (25) reported that fiber hypertrophy occurred in denervated Sol muscles with a low CL dose of only 10 μg · kg body wt⁻¹ · day⁻¹ whereas innervated Sol muscles required 200 μg · kg⁻¹ · day⁻¹ of CL before hypertrophy could be measured. Although speculative, it is also possible that the β₂-receptor density was greater in the atrophied Sol fibers of senescent rats if the loss of contractile elements exceeded the rate of β₂-receptor downregu-
tion, or, similar to denervation (1), atrophy may induce upregulation of β2-receptors. Furthermore, Larkin et al. (22) showed that β-receptor density increases in skeletal muscles without changes in adenylyl cyclase activity. Together, these findings suggest the elevated β2-receptor density in Sol muscles from old animals would provide a more favorable environment for binding the low physiological levels of CL, thereby delaying muscle protein loss, relative to muscles from young adult animals or compared with the Pl muscles of either age group. Thus the relatively greater effect of preserving contractile elements by CL during HS in slow muscles of senescent rats and therefore attenuating the loss of P0 may be due to the elevated β2-receptor density in slow muscles (38) of senescent vs. young adult rats. The modest effects of CL were selective for slow muscles, because, even though the Pl atrophied with HS in senescent rats, there was no evidence that this muscle derived any protein sparing from the physiological dose of CL. It is possible that, even if the receptor density is not downregulated in proportion with fiber size, there may be too few β-receptors in the Pl to induce a measurable change in myofibrillar sparing. The differences in response between Pl and Sol muscles (albeit small) at the physiological dose of CL may be explained by the greater β-receptor density in slow than fast muscles (38).

A second possibility is that CL and aging-associated changes in levels of myogenic transcription factors may favor preservation of contractile elements in slow muscles. It is known that muscles from senescent animals increase their expression of several myogenic transcription factors under control conditions, presumably as an attempt to maintain their muscle mass or delay the loss of muscle tissue during sarcopenia (27). In addition, myogenic regulator factor transcription is also elevated in muscles of old animals under conditions resulting in muscle hypertrophy (23, 24, 28). Myogenin is primarily found in slow muscles, whereas MyoD is found in abundance in fast muscles (20), but only myogenin is elevated after CL administration (14). It is therefore possible that the aging-enhanced expression of myogenin was further enhanced by physiological doses of CL in the slow Sol muscle, but MyoD, the primary transcription factor for the fast Pl muscle was not affected by CL. Further studies are needed to determine whether CL mediates its attenuation of protein loss in the Sol of old rats after HS, in part by elevating myogenin transcription.

The low dose of CL used in the present study appeared to be ineffective for attenuating non-weight-bearing losses of muscle force and muscle mass in Pl muscles, and it provided only modest changes in the slow Sol muscles of old rats. Although CL treatment resulted in increases in the relative expression of type IIX MHC in Sol muscles from young rats (Fig. 2), this did not influence Sol force production (Fig. 1) or muscle mass (Table 1). This finding contrasts with previous studies that have used supraphysiological doses of CL and reported anabolic effects and improvements in force production and muscle mass after CL use in both young and old rats (9, 10, 17). It is unlikely that the dissimilarity in results can be attributed solely to the differences between the strain of rats used in those studies and the Fischer 344 × Brown Norway rats used in our present study, but this possibility cannot be eliminated without further study. Rather, it is likely that the “safe clinical” dose CL (0.01 mg · kg body wt · day−1) that we used in this study was too low to induce large-scale changes in Pl muscle catabolism under non-weight-bearing conditions.

Our data support the conclusion that CL administered at physiological doses (0.01 mg · kg−1 · day−1) is largely an ineffective countermeasure for preventing muscle wasting from HS in senescence. Physiological levels of CL may modestly slow the amount of muscle atrophy in slow muscles of senescent rats, perhaps by taking advantage of the enhanced β-receptor density induced by denervation and atrophy, or by enhancing transcription of the myogenic regulator factor myogenin. Nevertheless, in senescent rats, ~83% of the Sol muscle CSA atrophy and force losses still occurred in the HS+CL group compared with the senescent HS+placebo group. Studies that report that CL is effective for elevating protein accumulation of skeletal muscle (9, 11, 12, 25) and also for slowing muscle atrophy (5, 6) have all used high levels of CL (e.g., 0.2–2.0 mg · kg−1 · day−1) that exceed equivalent predicted safe doses in humans. This β2-agonist would be potentially harmful if used in these high doses in the elderly (26), and, because it has no effect on fast muscles and only very modest effects on slow muscles, we consider CL to be an ineffective countermeasure for offsetting muscle sarcopenia and disuse-induced atrophy in senescence.

I thank Dr. K. D. Chen, who passed away after succumbing to cancer, for his contributions to this paper.

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