Diminished satellite cells and elevated adipogenic gene expression in muscle as caused by ovariectomy are averted by low-magnitude mechanical signals

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Frechette DM, Krishnamoorthy D, Adler BJ, Chan ME, Rubin CT. Diminished satellite cells and elevated adipogenic gene expression in muscle as caused by ovariectomy are averted by low-magnitude mechanical signals. J Appl Physiol 119: 27–36, 2015. First published April 30, 2015; doi:10.1152/japplphysiol.01020.2014.—Age-related degeneration of the musculoskeletal system, accelerated by menopause, is further complicated by increased systemic and muscular adiposity. The purpose of this study was to identify at the molecular, cellular, and tissue levels the impact of ovariectomy on adiposity and satellite cell populations in mice and whether mechanical signals could influence any outcomes. Eight-week-old C57BL/6 mice were ovariectomized, with one half subjected to low-intensity vibration (LIV; 0.3 g/90 Hz, 15 min/day, 5 day/wk; n = 10) for 6 wk and the others sham vibrated (OVX; n = 10). Data are compared with age-matched, intact controls (AC; n = 10). In vivo μCT analysis showed that OVX mice gained 43% total (P < 0.001) and 125% visceral adiposity (P < 0.001) compared with their baseline after 6 wk, whereas LIV gained only 21% total (P = 0.01) and 70% visceral adiposity (P < 0.01). Relative to AC, expression of adipogenic genes (PPARγ, FABP4, PPARδ, and FoxO1) was upregulated in OVX muscle (P < 0.05), whereas LIV reduced these levels (P < 0.05). Adipogenic gene expression was inversely related to the percentage of total and reserve satellite cell populations in the muscle, with both declining in OVX compared with AC (−21 and −28%, respectively, P < 0.01). LIV mitigated these declines (−11 and −17%, respectively). These results provide further evidence of the negative consequences of estrogen depletion and demonstrate that mechanical signals have the potential to interrupt subsequent adipogenic gene expression and satellite cell suppression, emphasizing the importance of physical signals in protecting musculoskeletal integrity and slowing the fat phenotype.

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stem cell fate toward muscle and away from fat may aid in retaining muscle quality. As a potential adjunct to exercise, mechanical stimulation in the form of low-intensity vibration (LIV; \(< 0.4\ g\), where \(g\) is earth’s gravitational field at 9.81 \(\text{m/s}^2\)) has been shown to provide a signal that is both anabolic (LIV; retaining muscle quality. As a potential adjunct to exercise, stem cell fate toward muscle and away from fat may aid in maintaining satellite cell populations that are likely to be negatively affected by the lack of E2 and/or secondary complications of ovariectomy.

This study tested the hypothesis that ovariectomy will compromise skeletal muscle satellite cell populations and increase abdominal adiposity and muscular adipogenic gene expression, whereas daily bouts of LIV would protect the muscle from increased adipogenic gene expression and muscle stem cell impairment. Using an ovariectomized (OVX) C57BL/6 murine model, we identified the effects of ovariectomy on satellite cell populations and adipogenic and myogenic gene expression within the skeletal muscle and determined whether LIV could mitigate this deterioration.

**MATERIALS AND METHODS**

**Experimental design.** All experimental procedures were reviewed and approved by Stony Brook University’s Institutional Animal Care and Use Committee. Eight-week-old female C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) received an ovariectomy (n = 20) or sham surgery (n = 10). Following 2 wk of recovery and acclimation, a weight-matched, randomized MATLAB script was used to divide the ovariectomized mice into two groups, OVX (n = 10) and OVX + vibration treatment (LIV; n = 10). Animals receiving a sham surgery were grouped as age-matched controls (AC; n = 10). Experimental treatment and baseline measurements began 2 wk post-surgery at 10 wk of age; this time point is considered the start of the experimental protocol (t = 0 wk). Six weeks following time zero, all 30 mice were anesthetized with isoflurane and euthanized via cervical dislocation (8 wk postsurgery, 16 wk of age; Fig. 1). End-point in vivo measurements were taken 2 days prior to euthanasia. Mice were single-housed at 21°C and fed a standard rodent chow diet (LabDiet 5013; Purina Mills, St. Louis, MO), with ad libitum access to food and water.

**Mechanical stimulation protocol.** Animals receiving mechanical stimulation treatment were subject to low-magnitude, high-frequency, vertically oscillating vibrations (0.3 g at 90 Hz). LIV was administered for 15 min/day, 5 day/wk for 6 wk. Mice were placed into a partitioned box that was centered on the vibration plate. AC and OVX were sham handled and placed in the same box, but on an inactive device. The last vibration treatment was administered the day prior to euthanasia.

**In vivo microcomputed tomography measurements.** Total abdominal adiposity was quantified in vivo at both baseline (t = 0 wk) and endpoint (t = 6 wk) using X-ray microcomputed tomography (μCT) (vivaCT 40; Scanco Medical, Brüttisellen, Switzerland). Animals were anesthetized with 2% isoflurane inhalation and stabilized in a custom-made, foam scanning bed. Scans of the abdomen (76 μm isotropic resolution, 45 kV intensity, 133 μA current) were evaluated from the L1–L5 vertebrae (~20-mm region). Visceral and subcutaneous adipose tissues in this region were segregated using a well-established segmentation script (38). Parameters that included visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), and total adipose tissue (TAT; subcutaneous + visceral) were reported by volume (mm³).

**Quantitative polymerase chain reaction of skeletal muscle gene expression.** Following euthanasia, the soleus muscle was excised and stored in RNAlater at 4°C and then moved to −20°C for long-term storage. RNA was isolated using a commercial spin kit for skeletal muscle (RNeasy Fibrous Tissue Mini Kit; Qiagen, Austin, TX). RNA purity was quantified using 1 μl of mRNA and a NanoDrop 1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). cDNA conversion was performed using a high-capacity reverse transcription kit (Applied Biosystems, Foster City, CA), and amplification was conducted using TaqMan Gene Expression Assays and TaqMan Gene Expression Master Mix (Applied Biosystems). Expression levels of genes critical to adipogenesis, including peroxisome proliferator-activated receptor (PPARγ), fatty acid-binding protein 4 (FABP4), PPARγ, forkhead box protein O1 (FoxO1), adiponectin (Adipoq), and those critical to myogenesis, including paired box protein 7 (Pax7), myoblast determination protein (MyoD), myogenin factor 5 (Myf5), insulin-like growth factor 1 (IGF-1), and myostatin (Mstn), were analyzed and compared with the housekeeping gene β-actin. Relative expression was compared against intact control animals using the ΔΔCT method of analysis.

**Flow cytometric analysis of satellite cells.** To evaluate satellite cell status in skeletal muscle, the left gastrocnemius and quadriceps muscles were harvested immediately following euthanasia, minced, pooled for each animal, and stored in Dulbecco’s modified essential medium (DMEM; Gibco, Carlsbad, CA) with 1% penicillin-streptomycin. Satellite cell isolation was adapted from a previous protocol (25), with changes as noted. Tissue fragments were digested with 2 mg/ml collagenase type II, 1.2 U/ml trypsin, and 2 mM CaCl₂ in PBS. After trituration, samples were neutralized with DMEM supplemented with 1% penicillin-streptomycin and 15% horse serum (Thermo Scientific). Mononuclear cells were filtered with 40 μm nylon cell strainers (BD Biosciences, San Diego, CA). Samples were centrifuged at 2,000 rpm for 5 min and resuspended in 1× lysis buffer (Pharm Lyse; BD Biosciences) for 5 min at room temperature. Cells were again centrifuged, resuspended in DEMEM supplemented with 1% penicillin-streptomycin, and counted; 1 × 10⁶ cells were removed from each sample for staining. Samples were incubated for 45 min in
adiponectin was elevated in OVX animals compared with AC (+79%, \( P < 0.05 \)), whereas the increase in LIV mice was not significantly different from AC (+72%, \( P > 0.05 \); Fig. 3C). No differences were detected between groups in the leptin/adi-
ponectin ratio (LAR) \( (P > 0.05) \); Fig. 3D), but these values were positively correlated to TAT volume \( (P < 0.01, r = 0.718; \text{Fig. } 3E)\).

Abdominal adiposity is increased by OVX, whereas LIV trends toward mitigated adipose accretion. At the study base-
line, 2 wk postovariectomy, and immediately before LIV, adipose distributions in both OVX and LIV groups were
significantly different from AC, whereas there was no difference in TAT at this point in time (Fig. 4B). VAT represented
19% of TAT in both OVX and LIV groups at baseline compared with 26% in AC \((P \leq 0.05)\), whereas SAT represented
81% of TAT in both OVX and LIV compared with 74% in AC \((P \leq 0.05)\).

Over the course of the study, OVX animals gained 43% in TAT \((P < 0.001)\), whereas LIV gained 21% \((P = 0.01)\) and
AC +7% \((\text{Fig. } 4B)\). The TAT percent change from baseline for each individual animal in OVX mice was 44% greater than AC
\((P < 0.05),\) whereas the 23% increase in mice subject to LIV was not significantly different from AC (Fig. 4C). After 6 wk,
both OVX and LIV animals had greater subcutaneous fat volumes \((658 \pm 124 \text{ and } 621 \pm 58 \text{ mm}^3, \text{respectively})\) com-
pared with AC \((507 \pm 93 \text{ mm}^3, P < 0.01 \text{ and } P < 0.05, \text{respectively})\). OVX animals indicated a trend toward greater
VAT volume compared with AC, with a 70% increase by the study conclusion \((P = 0.06)\), whereas LIV animals had only a
33% increase \((\text{Fig. } 4D)\). Compared with baseline, VAT accumulation increased in OVX animals 125% \((P < 0.001)\) com-
pared with 70% \((P < 0.01)\) with LIV, whereas control animals gained 1.4% VAT over this same period of time (Fig. 4D).

Ovariectomy increases adipogenic gene activity in muscle. PPAR\(\gamma\) is increased 120 and 78%, respectively, in the
soleus muscle of OVX compared with AC \((P \leq 0.001)\; \text{Fig. } 5)\). In contrast, expression levels of PPAR\(\delta\) increased by 27% and
PPAR\(\gamma\) decreased by 15% in LIV compared with AC \((P > 0.05),\) representing a differential expression of LIV relative to
OVX of −42 and −52%, respectively \((P \leq 0.001)\). Compared with AC, FABP4 was upregulated in OVX by 93% \((P <
0.001)\) and downregulated in LIV by 46% compared with OVX \((P < 0.001)\). Similarly, FoxO1 was upregulated by 113% in
OVX compared with AC \((P < 0.001)\), whereas LIV reduced

![Fig. 2](http://jap.physiology.org/)<br>

Fig. 2. A: body weights from week 0 (2 wk postovariectomy) through week 6. At time 0, differences in weight had already occurred due to the ovariectomy surgery 2 wk prior to study baseline. \(*P < 0.05\), OVX compared with age-matched controls (AC); \(*P < 0.05\) LIV compared with AC. B: soleus wet weights measured at euthanasia. \(*P < 0.05\) compared with AC. C: gonadal fat pad wet weights measured at euthanasia. \(*P < 0.05\) compared with AC.
this expression by 59% compared with OVX ($P < 0.001$). No differences in Adipoq expression levels were detected.

**OVX modulates myogenic gene expression in the soleus.** Pax7 and MyoD were upregulated in both the OVX and LIV mice compared with AC, with Pax7 up 89 ($P < 0.001$) and 70% ($P < 0.01$) and MyoD elevated by 74 ($P < 0.01$) and 60% ($P < 0.05$), respectively (Fig. 5). Expression levels of Myf5 were elevated in OVX compared with AC by 89% ($P < 0.001$), whereas LIV was 26% lower than OVX ($P < 0.05$). IGF-I was upregulated in OVX by 72% ($P < 0.05$) compared with AC, whereas changes in LIV (−28%, $P > 0.05$) were not significantly different from AC. Lastly, no differences were detected in Mstn expression levels.

**OVX compromises satellite cell populations.** Satellite cell populations measured in the pooled gastrocnemius and quadriceps muscles (Fig. 6, A and B), positive for integrin α7 and negative for markers CD31, CD45, and Sca-1 (25), were 21% lower in OVX compared with AC ($P < 0.01$; Fig. 6C). In contrast, there were no significant differences between AC and LIV (−11%, $P > 0.05$). This relationship is consistent in the proportion of reserve satellite cells in the muscle tissue, as indicated by the positive marker CD34 (25). OVX mice had a 28% reduction ($P < 0.01$) in percent CD34+ satellite cells compared with AC, whereas LIV mice had proportions reduced by 17% ($P > 0.05$; Fig. 6D). LIV was not different from OVX in either cell population. No differences were measured in the number of CD34− cells (data not shown). Additionally, total cell number was not different between any of the three groups, indicating true changes in satellite cell populations (data not shown).

**DISCUSSION**

Ovariectomy in the mouse was used to model phenotypic changes that follow menopause in the human, with a specific focus on the rapid escalation of systemic adiposity and changes in muscular composition that parallel estrogen deficiency. It is well established that ovariectomy in mice increases susceptibility to obesity (44, 51), and the data reported here indicate that the transformation in body habitus occurs within 2 wk following ovariectomy. Whereas estrogen depletion resulted in a higher average body mass of 11% compared with controls only 2 wk postsurgery, the following 6 wk resulted only in an additional 1.5% increase relative to controls. Certainly, when considering the consequences of ovariectomy on the mouse, the reality that this response happens so rapidly must be recognized. In retrospect, the introduction of the LIV signal, or any intervention, to this model beginning 2 wk postsurgery rather than immediately after surgery would appear to constitute more of a test of “reversal of” rather than “protection from” the impact of OVX.

LIV has previously been shown to suppress adipogenesis in murine models of diet-induced obesity (40, 54), and the OVX data presented here indicate trends toward this mitigation. However, when comparing gonadal fat pad weights and abdominal adiposity as quantified by μCT, the data suggest that LIV continues to suppress adiposity despite no effects on total...
body mass. Whereas both OVX and LIV mice had significant increases in adiposity from baseline (2 wk following OVX), TAT and VAT volume increases in LIV mice at the conclusion of 6 wk were approximately one-half of that measured in the OVX group. And certainly, the challenges of catabolizing extant adipose tissue that existed after 2 wk are different from that of suppressing the formation of fat.

The idea that adipogenesis is being mitigated by these low-level mechanical signals is further supported by differences in serum adipokine levels measured at the end of protocol. Circulating leptin levels were 220% higher in OVX relative to age-matched controls, similar to previously published trends (26), and may reflect metabolic abnormalities related to excessive adipose tissue. In some contrast, no significant differences in leptin levels were measured in LIV mice compared with controls. Circulating adiponectin concentrations showed similar trends. Expressing leptin and adiponectin as a ratio can be indicative of insulin resistance and may be a better indication of adipocyte or metabolic health than solely leptin or adiponectin (7, 13, 45, 56). However, the data reported here show no differences in LAR despite a relatively strong correlation to TAT. These data indicate that mechanical signals represent a reasonable means to control adipokine levels even in the face of systemic pressures to become imbalanced.

The systemic increase in adipose burden that followed ovariectomy, measured as increased fat depots in the subcutaneous and visceral compartments, was also realized locally in the musculature, as reflected by the elevation of adipogenic gene expression measured in the soleus. Regulators of adipogenesis, including PPARγ, PPARδ, FABP4, and FoxO1, were significantly upregulated in muscle from OVX mice, suggesting that despite a conservation of mass, the quality of the muscle was

Fig. 4. A: In vivo microcomputed tomography 3-dimensional reconstructions (76-μm resolution) of transverse abdominal sections depicting end-point (after 6 wk of LIV treatment) TAT. B: TAT volume; %increases shown are the differences between the group’s average TAT volume at baseline vs. the group’s end-point volume. C: TAT volume %change was calculated for each individual animal from its own baseline value, all of which were used to obtain a group average. D: VAT volume. *P < 0.05 compared with AC at the corresponding time point. #P < 0.05 compared with baseline measurement.
deteriorating. Increases in the PPAR genes indicate alterations in fatty acid metabolism (39), an outcome supported by the upregulation of FABP4. The measured increases in PPARγ expression, a major regulator of adipocyte differentiation, are likely linked to increased fatty acid transport and binding in the muscle tissue. Fatty acids bind with high affinity to fatty acid-binding proteins such as FABP4, which are upregulated as a result of both fatty acid transport and elevated PPARγ expression. PPARδ is also activated by fatty acids and plays a role in preadipocyte proliferation (27), which activates PPARγ and may ultimately contribute to an overall increase in adipocyte encroachment into the muscle. Transcription factor FoxO1 is downstream of PPARδ and plays a role in the mediation of oxidative metabolism and preadipocyte differentiation. Paired regulation of PPARδ and FoxO1 expression levels have been reported in the OVX model, although both genes were downregulated (50). Differences in expression trends are possibly due to specific muscle selection (soleus vs. quadriceps) and differing fiber compositions. No differences were measured in Adipoq, an ant adipogenic gene that is associated with decreased skeletal muscle triglyceride content in mice (69) and increases fatty acid oxidation in skeletal muscle cells (71).

That the marked elevations in circulating adipokine concentrations following ovariectomy were mirrored by elevated adipogenic gene expression in the muscle tissue implicates the depletion of ovarian hormones in the decline of multiple tissue systems. Whether the noted increase in intramuscular adiposity is a result of adipocyte encroachment or adipogenic differentiation of preexisting stem cells within the muscle tissue requires further investigation. Furthermore, although these genes are known to participate in adipogenesis, all are involved in other skeletal muscle metabolic processes such as muscular mitochondrial biogenesis and glucose metabolism (43); changes in expression may have additional influences on the muscle tissue.

High levels of intramuscular adiposity are associated with both aging (6) and obesity (19); however, an “athlete’s paradox” that demonstrates similarly elevated skeletal muscle adiposity in well-trained endurance athletes exists (18). Fat is a key regulator of many physiological processes (2, 4, 60) and increased intramuscular fat may not translate to decreased function. In this particular case, a buildup of fat in the muscle due to ovarian hormone depletion is likely to put muscle function at risk (62), including a reduction in muscular strength (17), which intrinsically plays a role in functional performance and contractile properties (47). In some contrast to the adipogenic bias measured in the muscle of OVX, there was a significant suppression (>40%) of those parameters in mice subjected to LIV, suggesting that these mechanical signals provided some form of a protective mechanism against adipogenesis that may ultimately lead to a reduction in the number of intramuscular adipocytes and the protection of muscle quality. Because exercise is known to reduce adiposity and is often prescribed to postmenopausal women to help maintain muscular integrity, it is possible that the mechanical signals derived from LIV serve as a form of exercise surrogate to provide similar salutary benefit. Considering that LIV has been shown in humans to protect postural stability (42) and reduce falls in the elderly (36), it is possible that these clinical outcomes are achieved by retaining both quality and neuromuscular control of the muscle (41).

Increases in intramuscular adiposity will invariably disrupt the muscle microniche, ultimately encroaching upon satellite cell populations and thus impacting the ability for repair and regeneration of muscle tissue. Indeed, in parallel with the increased adipogenic activity of muscle in OVX, there was a significant decrease in the proportions of satellite cells compared with total cells in the muscle. It has been noted that CD34 expression on satellite cells is a reversible state of activation that influences stem cell quiescence. CD34+ satellite cells have been identified as a reserve population of satellite cells that divide early on in response to injury. These cells will eventually become CD34− for later proliferation involved in the reparative process (25). The proportion of CD34+ cells in OVX was reduced compared with controls, which may suggest that the immediate ability of the muscle to respond to injury or demands for myogenesis is limited. The simultaneous increase in local adipogenic gene expression may be what is driving the reduction of satellite cells and/or CD34+ satellite cells through biased stem cell differentiation or disrupted muscle homeostasis.

Although LIV did not have higher satellite cell proportions compared with OVX, no differences in overall satellite cell proportion or CD34+ satellite cells were observed in LIV compared with age-matched controls, suggesting that these mechanical signals served to protect these progenitors. Preserving the CD34+ reserve population of satellite cells may be critical in maintaining muscle quality and quantity during a progressive loss of ovarian hormone. Mechanical signals delivered in the form of exercise or, when that is not possible, perhaps as LIV may play a critical role in the regeneration, rehabilitation, and restoration of function of muscle tissue.

Myogenic gene expression influences the proliferation and differentiation of satellite cells. Proliferating satellite cells simultaneously express Pax7 and MyoD (23), both of which were upregulated in OVX. Whereas satellite cell proliferation and differentiation genetic markers were increased, the number of satellite cells in the OVX group was lower than that of controls. Although the actual proportion of satellite cells in the
muscle is compromised, it appears that OVX is responding to injury by upregulating myogenic gene expression to repair overall muscle homeostasis. MyoD, a gene also expressed by myocytes, was also upregulated, possibly because of the initiation of proliferating muscle cells. Myf5 promotes satellite cell renewal and myoblast differentiation and was again upregulated in OVX animals, perhaps as another effort to repair and regenerate damaged muscle. IGF-I was also upregulated in the OVX group. Overexpression of IGF-I stimulates muscle hypertrophy (5) through satellite cell activation and the upregulation of protein synthesis (24) during active postnatal muscle development or adult regeneration (57). Both OVX and LIV groups had heavier soleus weights at euthanasia, consistent with heavier muscle weights found in other OVX murine models (10, 14, 61). There were no changes in the expression of Mstn, which is responsible for preventing muscle growth (32).

LIV mice also had elevated levels of Pax7 and MyoD, an indication that despite the exercise surrogate, the muscle tissue was still signaling the process of repair. Although mechanical stimulation is generally found to increase the expression of myogenic factors (67, 70), there was a significant decrease in Myf5 expression in LIV relative to OVX. Myf5 is one of the earliest markers of active satellite cell commitment to the myogenic lineage. The noted changes of Myf5 in the LIV group may be a result of the transition of stem cells into a myoblast differentiation phase where Myf5 is consequently downregulated (33). Overall, LIV resulted in less of an impact on myogenic markers than those related to adipogenesis, which suggests that the primary influence of LIV is associated with suppressing adiposity rather than influencing myogenesis.

Our gene expression profiles consisted of chiefly anabolic genes, which we saw respond toward an adipogenic profile. Certainly, a concurrent assessment of catabolic genes would give a more complete analysis of the effects of LIV on OVX skeletal muscle tissue. Although two catabolic genes in this study were assessed (adiponectin and myostatin), no differences in their expression profile were detected between any groups. Clearly, the systemic insult of OVX is significant, and the mechanisms involved in the consequences, and the mechanical protection, are certain to be complex. Ultimately, a more comprehensive transcriptional profile must be performed before a more complete picture is possible.

The reported changes in gene expression due to OVX and vibration treatment cannot be attributed solely to one cell population and rather represent variations across the muscular homeostasis.
environment. Changes in the stem cell niche may be due to a
number of cell types involved in skeletal muscle regeneration
and maintenance, e.g., fibroblasts, endothelial cells, fibro/adipogenic progenitors, telocytes, motor neurons, and mesenchymal
and hematopoietic stem cells and precursors, and are likely
to influence satellite cell activity. Additionally, although all
muscles used for this study were extracted from the hindlimbs,
muscle groups differed among assays due to assay cell number
and tissue mass requirements. Variations in gene expression
and satellite cell numbers may occur, depending on anatomic
location. Further investigation into the mechanism behind
OVX’s impact and LIV’s salutary influence on adipogenesis
and myogenesis, in addition to local variations in the skeletal
muscle, is necessary. It is also important to note that future
studies must include the assessment of muscular composition
on the phenotypic level (lipid content, adipocyte infiltration,
etc.) and functional testing to demonstrate changes in muscular
strength and quality.

The work presented here provides evidence for the rapid and
marked consequences of ovariectomy on a number of physio-
logical systems. In particular, this work shows that the removal
of ovarian hormones increases adipogenic gene expression in
muscle, reducing its quality, and simultaneously suppresses the
number of satellite cells available for muscle regeneration and
repair. These data also indicate that extremely low-level me-
chanical signals, introduced for brief periods each day using
LIV, reduce levels of total adiposity and suppress muscular
adipogenic gene expression while protecting the number of
satellite cells.

Exercise, representing the primal mechanical stimulus, is a
critical modality to slow the range of complications that arise
from menopause, including the deterioration of muscle quality
and strength. Ironically, however, the menopause and its se-
quelae, such as the rapid decline of the musculoskeletal system,
can erode the safety, ease, and compliance required of exercise.
Although there is no true substitute for exercise, the data
presented here suggest that LIV may have the potential to serve
as a surrogate for exercise for the injured or impaired. Ulti-
mately, alternatives to exercise, either chemical or physical,
that help retain the progenitor population and its microniche
sooner rather than later may help preserve the integrity of a
range of physiological systems.

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DISCLOSURES

C. T. Rubin is a founder of Marodyne Medical. All other authors declare no potential conflicts of interest.

AUTHOR CONTRIBUTIONS


REFERENCES

244, 1993.
6. Cree MG, Newcomer BR, Katsanos CS, Sheffield-Moore M, Chinkes D, Aarsland A, Urban R, Wolfe RR. Intramuscular and liver triglyc-
8. Csete M, Walkonis J, Slavny N, Wei YW, Korsnes S, Doyle JC, Wold B. Oxygen-mediated regulation of skeletal muscle satellite cell prolifera-
10. Dalgreviren S, Kandile HB, Uysal B, Zebek ND, Korkusuz P, Gu-
musel B, Korkusuz F. Tumor necrosis factor-alpha antagonist adminis-
20. Greeves JP, Cable NT, Reilly T, Kingsland C. Changes in muscle strength in women following the menopause: a longitudinal assessment of...


36. Sitnick M, Foley AM, Brown M, Spangenburg EE. Expression and splicing of the insulin-like growth factor 1 and myostatin in ovarioectomy during myogenesis in the posthatch chicken establishes a model for muscle satellite cell proliferation and differentiation promotes osteogenesis while preventing dietary-induced CT. *Biochim Biophys Acta* 1795, 2014.

37. Smith GI, Sinacore DR, Shah K, Mittendorfer B. The ratio of skeletal muscle insulin-like growth factor 1 and myostatin in ovarioectomy during myogenesis in the posthatch chicken establishes a model for muscle satellite cell proliferation and differentiation promotes osteogenesis while preventing dietary-induced CT. *Biochim Biophys Acta* 1795, 2014.


48. Smith GI, Sinacore DR, Shah K, Mittendorfer B. The ratio of skeletal muscle insulin-like growth factor 1 and myostatin in ovarioectomy during myogenesis in the posthatch chicken establishes a model for muscle satellite cell proliferation and differentiation promotes osteogenesis while preventing dietary-induced CT. *Biochim Biophys Acta* 1795, 2014.


