Mechanical overload and skeletal muscle fiber hyperplasia: a meta-analysis

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Kelley, George. Mechanical overload and skeletal muscle fiber hyperplasia: a meta-analysis. J. Appl. Physiol. 81(4): 1584–1588, 1996.—With use of the meta-analytic approach, the purpose of this study was to examine the effects of mechanical overload on skeletal muscle fiber number in animals. A total of 17 studies yielded 37 data points and 360 subjects met the initial inclusion criteria: 1) “basic” research studies published in journals, 2) animals (no humans) as subjects, 3) control group included, 4) some type of mechanical overload (stretch, exercise, or compensatory hypertrophy) used to induce changes in muscle fiber number, and 5) sufficient data to accurately calculate percent changes in muscle fiber number. Across all designs and categories, statistically significant increases were found for muscle fiber number [15.00 ± 19.60% (SD), 95% confidence interval = 8.65–21.53], muscle fiber area (31.60 ± 44.30%, 95% confidence interval = 16.83–46.37), and muscle mass (90.50 ± 86.50%, 95% confidence interval = 61.59–119.34). When partitioned according to the fiber-counting technique, larger increases in muscle fiber number were found by using the histological vs. nitric acid digestion method (histological = 20.70%, nitric acid digestion = 11.10%; P = 0.14). Increases in fiber number partitioned according to species were greatest among those groups that used an avian vs. mammalian model (avian = 20.95%, mammalian = 7.97%; P = 0.07). Stretch overload yielded larger increases in muscle fiber number than did exercise and compensatory hypertrophy (stretch = 20.95%, exercise = 11.59%, compensatory hypertrophy = 5.44%; P = 0.06). No significant differences between changes in fiber number were found when data were partitioned according to type of control (intra-animal = 15.20%, between animal = 13.90%; P = 0.82) or fiber arrangement of muscle (parallel = 15.80%, pennate = 11.60%; P = 0.61). The results of this study suggest that in several animal species certain forms of mechanical overload increase muscle fiber number.

muscle mass; enlargement; hypertrophy

RECENTLY, A NARRATIVE REVIEW has suggested that increases in muscle fiber number (hyperplasia) in animals occur as a result of stretch overload, whereas compensatory hypertrophy (ablation, tenotomy) does not generally change fiber number (8). In addition, it was also reported that exercise models in animals have led to mixed results with regard to increases in muscle fiber number (8). Although the above-mentioned review provided valuable information, it relied on the traditional narrative approach, that is, chronologically arranging and then describing studies. A need exists for the quantification of the magnitude and direction of changes in skeletal muscle fiber number as a result of different types of mechanical overload in animals. Thus the purpose of this study was to use the meta-analytic approach (12, 14, 20, 26) to examine the effect of different types of mechanical overload (stretch, exercise, and compensatory hypertrophy) on skeletal muscle fiber number in animals.

METHODS

Literature search. The search for literature was limited to studies published in journals between January 1966 and December 1994. Studies in English-language journals were obtained from computer searches (Medline) as well as hand searches and cross-referencing. The search for studies in foreign-language journals was limited to computer searches (Medline) only. Specific inclusion criteria were 1) “basic” research studies published in journals, 2) animals (no humans) as subjects, 3) control group (intra- or between animal) included, 4) some type of mechanical overload employed (stretch, exercise, compensatory hypertrophy), and 5) sufficient data to calculate percent changes in muscle fiber number. Human studies were not included in this analysis for two reasons: 1) only one study providing quantitative data on humans is known to exist and 2) the methods used to examine muscle fiber number in humans are not as accurate as in animals (29).

Recording and classifying variables. All studies that met the criteria for inclusion were recorded on a recording sheet (available on request) that could hold up to 81 pieces of information. The major categories of information recorded included 1) study characteristics (year, journal, length of study, number of groups, number of subjects, type of study, i.e., intra-animal or between animal, and muscle examined), 2) physical characteristics of subjects (type of animal, age, weight, and diet), 3) mechanical overload characteristics (length, frequency, intensity, duration, and mode), and 4) skeletal muscle changes (muscle mass, muscle fiber area, and muscle fiber number). To avoid bias in selecting and rejecting studies, the decision to include a paper was made by examining the methods and results sections separately under coded conditions. A control group was defined as that group that did not receive any type of mechanical overload during the study. Two primary types of information were desired from the studies: outcomes and major variables that could affect outcomes. For this study, the major outcome was change in skeletal muscle fiber number. In addition, changes in muscle mass and fiber area were also examined. Major variables that could potentially affect changes in fiber number included 1) fiber-counting technique used (histological analysis vs. nitric acid digestion), 2) type of mechanical overload employed (stretch, exercise, or compensatory hypertrophy), 3) species used (avian vs. mammalian), 4) type of control (intra- vs. between animal), and 5) fiber arrangement of muscle (pennate vs. parallel).

Statistical analysis. In a meta-analysis, the mean results for each group from each study are recorded irrespective of whether or not the results from each study are statistically significant. For this study, descriptive statistics (percentages) were used to report changes in muscle fiber number as well as changes in muscle fiber area and mass. Percentages were calculated by dividing the treatment minus control group difference by the control group value. Ninety-five percent confidence intervals were then established for each of the three major outcome variables, i.e., fiber number, fiber area, and muscle mass. Because there was no relationship between number of subjects and changes in skeletal muscle, no
weighting procedures were employed. Graphic analysis (Tukey box plots) were used to identify outliers. Individual outliers were then examined to justify whether there was any physiological justification for their removal from the analysis. Assessment of publication bias (the tendency for journals to publish studies that yield positive results) was not performed because the current statistical procedures addressing this issue lack validity (26).

Differences between changes in muscle fiber number and fiber area were examined by using a Mann-Whitney rank-

Table 1. Study characteristics

<table>
<thead>
<tr>
<th>Reference</th>
<th>Overload</th>
<th>Subject</th>
<th>Muscle</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alway (1)</td>
<td>Chronic stretch</td>
<td>Quail</td>
<td>ALD</td>
<td>NAD</td>
</tr>
<tr>
<td>Alway (2)</td>
<td>Chronic stretch</td>
<td>Quail</td>
<td>ALD</td>
<td>Histo</td>
</tr>
<tr>
<td>Alway (3)</td>
<td>Chronic stretch</td>
<td>Quail</td>
<td>ALD</td>
<td>Histo</td>
</tr>
<tr>
<td>Alway et al. (4)</td>
<td>Chronic stretch</td>
<td>Quail</td>
<td>ALD</td>
<td>NAD</td>
</tr>
<tr>
<td>Alway et al. (5)</td>
<td>Chronic stretch</td>
<td>Quail</td>
<td>ALD</td>
<td>NAD and Hist</td>
</tr>
<tr>
<td>Antonio and Gonyea (6)</td>
<td>Intermittent stretch</td>
<td>Quail</td>
<td>ALD</td>
<td>Histo</td>
</tr>
<tr>
<td>Antonio and Gonyea (7)</td>
<td>Intermittent stretch</td>
<td>Quail</td>
<td>ALD</td>
<td>Histo</td>
</tr>
<tr>
<td>Antonio and Gonyea (9)</td>
<td>Intermittent stretch</td>
<td>Quail</td>
<td>ALD</td>
<td>Histo</td>
</tr>
<tr>
<td>Gollnick et al. (15)</td>
<td>Chronic stretch</td>
<td>Chicken</td>
<td>ALD</td>
<td>NAD</td>
</tr>
<tr>
<td>Gollnick et al. (16)</td>
<td>Ablation</td>
<td>Rat</td>
<td>Soleus, plantaris, and EDL</td>
<td>NAD</td>
</tr>
<tr>
<td>Gonyea (17)</td>
<td>Weights</td>
<td>Cat</td>
<td>FCR</td>
<td>Histo</td>
</tr>
<tr>
<td>Gonyea (18)</td>
<td>Weights</td>
<td>Cat</td>
<td>FCR</td>
<td>Histo</td>
</tr>
<tr>
<td>Gonyea et al. (19)</td>
<td>Weights</td>
<td>Cat</td>
<td>FCR</td>
<td>NAD</td>
</tr>
<tr>
<td>Ho et al. (21)</td>
<td>Weights</td>
<td>Rat</td>
<td>AL</td>
<td>Histo</td>
</tr>
<tr>
<td>Tamaki et al. (28)</td>
<td>Sprints/weights</td>
<td>Rat</td>
<td>Plantaris</td>
<td>NAD</td>
</tr>
<tr>
<td>Timson et al. (30)</td>
<td>Ablation</td>
<td>Mouse</td>
<td>Soleus</td>
<td>NAD</td>
</tr>
<tr>
<td>Vaughan and Goldspink (31)</td>
<td>Tenotomy</td>
<td>Mouse</td>
<td>Soleus</td>
<td>Histo</td>
</tr>
</tbody>
</table>

ALD, anterior latissimus dorsi; EDL, extensor digitorum longus; FCR, flexor carpi radialis; AL, adductor longus; Histo, histological cross sections; NAD, nitric acid digestion.

Table 2. Changes in muscle fiber number for individual studies

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of Subjects</th>
<th>Treatment</th>
<th>Control</th>
<th>Difference</th>
<th>Change, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alway (1)</td>
<td>5</td>
<td>1,653 ± 239</td>
<td>1,278 ± 145</td>
<td>375</td>
<td>29</td>
</tr>
<tr>
<td>Alway (2)</td>
<td>15</td>
<td>1,764 ± 221</td>
<td>1,208 ± 128</td>
<td>556</td>
<td>46</td>
</tr>
<tr>
<td>Alway (3)</td>
<td>12</td>
<td>1,766 ± 343</td>
<td>1,189 ± 270</td>
<td>577</td>
<td>48</td>
</tr>
<tr>
<td>Alway et al. (4)</td>
<td>10</td>
<td>1,251 ± 328</td>
<td>1,200 ± 367</td>
<td>51</td>
<td>4</td>
</tr>
<tr>
<td>Alway et al. (5)</td>
<td>9</td>
<td>1,247 ± 315</td>
<td>1,143 ± 304</td>
<td>104</td>
<td>9</td>
</tr>
<tr>
<td>Alway et al. (5)</td>
<td>8</td>
<td>1,240 ± 253</td>
<td>1,154 ± 148</td>
<td>86</td>
<td>7</td>
</tr>
<tr>
<td>Alway et al. (5)</td>
<td>8</td>
<td>1,247 ± 335</td>
<td>1,084 ± 202</td>
<td>162</td>
<td>15</td>
</tr>
<tr>
<td>Alway et al. (5)</td>
<td>9</td>
<td>1,283 ± 228</td>
<td>1,024 ± 176</td>
<td>258</td>
<td>25</td>
</tr>
<tr>
<td>Alway et al. (5)</td>
<td>9</td>
<td>1,305 ± 304</td>
<td>999 ± 167</td>
<td>306</td>
<td>31</td>
</tr>
<tr>
<td>Antonio and Gonyea (6)</td>
<td>12</td>
<td>1,945 ± 419</td>
<td>1,281 ± 287</td>
<td>664</td>
<td>52</td>
</tr>
<tr>
<td>Antonio and Gonyea (7)</td>
<td>7</td>
<td>1,626 ± 188</td>
<td>1,652 ± 251</td>
<td>–26</td>
<td>–1</td>
</tr>
<tr>
<td>Antonio and Gonyea (9)</td>
<td>5</td>
<td>–10</td>
<td>–10</td>
<td>–10</td>
<td></td>
</tr>
<tr>
<td>Antonio and Gonyea (9)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Antonio and Gonyea (9)</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Gollnick et al. (15)</td>
<td>12</td>
<td>4,216 ± 575</td>
<td>4,116 ± 821</td>
<td>100</td>
<td>24</td>
</tr>
<tr>
<td>Gollnick et al. (16)</td>
<td>11</td>
<td>2,914 ± 282</td>
<td>2,910 ± 268</td>
<td>4</td>
<td>0.1</td>
</tr>
<tr>
<td>Gollnick et al. (16)</td>
<td>10</td>
<td>11,521 ± 715</td>
<td>11,481 ± 721</td>
<td>40</td>
<td>3.3</td>
</tr>
<tr>
<td>Gollnick et al. (16)</td>
<td>11</td>
<td>5,322 ± 58</td>
<td>5,254 ± 102</td>
<td>–22</td>
<td>–0.4</td>
</tr>
<tr>
<td>Gonyea (17)</td>
<td>5</td>
<td>9,081 ± 1,027</td>
<td>7,609 ± 918</td>
<td>1,472</td>
<td>19</td>
</tr>
<tr>
<td>Gonyea (18)</td>
<td>6</td>
<td>39,759 ± NR</td>
<td>36,550 ± NR</td>
<td>3,209</td>
<td>9</td>
</tr>
<tr>
<td>Gonyea et al. (19)</td>
<td>6</td>
<td>9,055 ± 1,029</td>
<td>7,522 ± 570</td>
<td>1,533</td>
<td>20</td>
</tr>
<tr>
<td>Ho et al. (21)</td>
<td>4</td>
<td>7,817 ± 810</td>
<td>7,556 ± 854</td>
<td>261</td>
<td>3</td>
</tr>
<tr>
<td>Tamaki et al. (28)</td>
<td>8</td>
<td>12,559 ± 269</td>
<td>11,030 ± 304</td>
<td>1,529</td>
<td>14</td>
</tr>
<tr>
<td>Timson et al. (30)</td>
<td>18</td>
<td>958 ± 92</td>
<td>953 ± 85</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>Timson et al. (30)</td>
<td>24</td>
<td>784 ± 220</td>
<td>798 ± 82</td>
<td>–14</td>
<td>2</td>
</tr>
<tr>
<td>Vaughan and Goldspink (31)</td>
<td>24</td>
<td>933 ± 188</td>
<td>752 ± 92</td>
<td>1,881</td>
<td>24</td>
</tr>
<tr>
<td>Vaughan and Goldspink (31)</td>
<td>24</td>
<td>990 ± 144</td>
<td>749 ± 193</td>
<td>241</td>
<td>32</td>
</tr>
</tbody>
</table>

Values for treatment and control are means ± SD. NR, not recorded.
Differences between changes in muscle fiber number partitioned according to potentially confounding variables (fiber-counting technique, species used, fiber arrangement of muscles, and type of control) were also examined by using Mann-Whitney rank-sum tests. A one-way analysis of variance test (Kruskal-Wallis) was used to examine the effect of different types of mechanical overload (stretch, exercise, and compensatory hypertrophy) on muscle fiber number. All data were reported as means ± SD. The significance level was set at $P < 0.05$.

**RESULTS**

Literature search. A total of 17 studies yielding 37 data points (some studies had >1 group) and 360 subjects met the initial criteria for inclusion (1–7, 9, 15–19, 21, 28, 30–31). Two quantitative studies (27, 33) were excluded because of insufficient information needed to accurately calculate percent changes in muscle fiber number. Another eight studies (10–11, 13, 22–25, 32) were excluded because only qualitative information was provided on muscle fiber number.

Study characteristics. A summary of study characteristics is given in Table 1. More studies (~53%) used chronic or intermittent stretch vs. exercise or compensatory hypertrophy (ablation, tenotomy) as the form of mechanical overload. Approximately 47% of the studies used the quail to examine muscle fiber hyperplasia while ~53% examined the anterior latissimus dorsi.
muscle for increased skeletal muscle fiber number. All of the studies used nitric acid digestion and/or histologi-
cross sections to assess changes in muscle fiber
number.
Changes in skeletal muscle. Changes in muscle fiber
number for individual studies are given in Table 2. Across all designs and categories, significant increases
in muscle mass (90.50 ± 86.50%, 95% confidence inter-
val = 61.59–119.34), fiber area (31.60 ± 44.30%, 95% confidence interval = 16.83–46.37), and fiber number
(15.00 ± 19.60%, 95% confidence interval = 16.83–46.37) were found (Fig. 1). Examination of out-
lier groups revealed no physiological reason to exclude
them from the analysis. Increases in fiber area were
approximately twice as great as increases in muscle
group. Isometric vs. isokinetic overload resulted in
increases in muscle fiber number. All speciesowered
somuchasthefactthatstretchwasthemechani-
ical overload employed on all avian species included in
this meta-analysis. The fact that increases in fiber
number were approximately twice as great when histo-
ological vs. nitric acid digestion methods were used is
consistent with previous investigations (5, 6). Because
of the ability to directly count each fiber, the nitric acid
digestion method is generally considered to be the more
accurate method of assessing changes in fiber number.
However, small fibers may be missed when this method
is used (8).

Despite the knowledge that studies can be more
objectively evaluated by using the meta-analytic vs.
traditional narrative approach, potential limitations
still exist. In general, the very nature of meta-analysis
dictates that the meta-analysis itself inherits those
limitations that exist in the literature. For example, a
review article by Timson (29) led him to conclude that
none of the animal models (stretch, exercise, or compen-
satory hypertrophy) currently used to examine exercise-
induced muscle enlargement truly represents the hu-
man strength-training situation under all conditions.
In addition, the fact that 11 of the 17 studies involved
essentially the same authors could have resulted in
biased results. In summary, the results of this study
suggest that in several animal specics certain forms of
mechanical overload increase muscle fiber number.

This meta-analysis attempted to quantify the magni-
tude of change in muscle (particularly muscle fiber
number) as a result of mechanical overload. Across all
designs and categories, mechanical overload resulted
in increases in muscle mass, muscle fiber area (hy-
ertrophy), and muscle fiber number (hyperplasia). Not
surprisingly, increases in fiber area were approxi-
mately twice as great as increases in fiber number. It
appears that hyperplasia in animals is greatest when
certain types of mechanical overload, particularly
stretch, are applied. The results of this investigation
are similar to a recent narrative review that concluded
that muscle fiber hyperplasia 1) consistently occurs as
a result of chronic stretch, 2) rarely occurs with over-
load in the form of compensatory hypertrophy, and 3)
has produced mixed results when overload in the form
of exercise is employed (8). Although it is well estab-
lished that mechanical-overload training results in
increased fiber area (hypertrophy), and thus increases
in muscle mass, the contribution of increased fiber
number (hyperplasia) to increases in muscle mass has
been more controversial. However, there now exists
quantitative evidence to support the fact that certain

types of overload, particularly stretch, result in in-
creases in muscle fiber number. Unfortunately, it is
beyond the scope of this investigation to examine the
processes (satellite cell proliferation and longitudinal
fiber splitting) responsible for such changes. The greater
changes in muscle fiber number found in avian vs.
mammalian species may not be the result of the species
used so much as the fact that stretch was the mechani-
cal overload employed on all avian species included in
this meta-analysis. The fact that increases in fiber
number were approximately twice as great when histo-
logical vs. nitric acid digestion methods were used is
consistent with previous investigations (5, 6). Because
of the ability to directly count each fiber, the nitric acid
digestion method is generally considered to be the more
accurate method of assessing changes in fiber number.

DISCUSSION

The author thanks Dr. Russ Moore (Dept. of Kinesiology, University of Colorado, Boulder, CO), Dr. Ben Timson (Dept. of Biomedical Science, Southwest Missouri State University, Springfield, MO), and Dr. Zung Vu Tran (College of Health and Human Sciences, University of Northern Colorado, Greeley, CO) for their assistance in the preparation of this manuscript.

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Received 26 February 1996; accepted in final form 15 February 1996.

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