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 yielding 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 changes 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

Table 1. *Study characteristics*

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

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

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 2. *Changes in muscle fiber number for individual studies*

Reference	No. of Subjects	Treatment	Control	Difference	Change, %
Alway (1)	5	1,653 ± 239	1,278 ± 145	375	29
Alway (2)	15	1,764 ± 221	1,208 ± 128	556	46
Alway (3)	12	1,766 ± 343	1,189 ± 270	577	48
Alway et al. (4)	10	1,251 ± 328	1,200 ± 367	51	4
	9	1,247 ± 315	1,143 ± 304	104	9
	8	1,240 ± 253	1,154 ± 148	86	7
	8	1,247 ± 335	1,084 ± 202	162	15
	8	1,283 ± 228	1,024 ± 176	258	25
	9	1,305 ± 304	999 ± 167	306	31
	9	1,462 ± 136	1,174 ± 102	287	24
Alway et al. (5)	12	1,945 ± 419	1,281 ± 287	664	52
Antonio and Gonyea (6)	7	1,626 ± 188	1,652 ± 251	−26	−1
Antonio and Gonyea (7)	5				−10
	5				0
	6				2
	5				31
	5				82
Antonio and Gonyea (9)	6	1,500 ± 148	1,631 ± 286	−131	−8
	6	1,803 ± 279	1,398 ± 210	405	29
Gollnick et al. (15)	12	4,216 ± 575	4,116 ± 821	100	24
Gollnick et al. (16)	11	2,914 ± 192	2,942 ± 192	−28	−1
	15	10,526 ± 1,359	10,564 ± 1,139	−38	−0.4
	5	5,224 ± 273	5,192 ± 74	32	0.6
	11	2,914 ± 282	2,910 ± 268	4	0.1
	10	11,521 ± 715	11,481 ± 721	40	0.3
	4	5,232 ± 58	5,254 ± 102	−22	−0.4
Gonyea (17)	5	9,081 ± 1,027	7,609 ± 918	1,472	19
Gonyea (18)	6	39,759 ± NR	36,550 ± NR	3,209	9
Gonyea et al. (19)	6	9,055 ± 1,029	7,522 ± 570	1,533	20
	4	7,817 ± 810	7,556 ± 854	261	3
Ho et al. (21)	15	2,477 ± 424	2,204 ± 530	273	12
Tamaki et al. (28)	8	12,559 ± 269	11,030 ± 304	1,529	14
	8	11,349 ± 327	11,030 ± 304	319	3
Timson et al. (30)	18	958 ± 92	953 ± 85	5	0.5
	24	784 ± 220	798 ± 82	−14	2
Vaughan and Goldspink (31)	24	933 ± 188	752 ± 92	1,881	24
	24	990 ± 144	749 ± 193	241	32

Values for treatment and control are means ± SD. NR, not recorded.

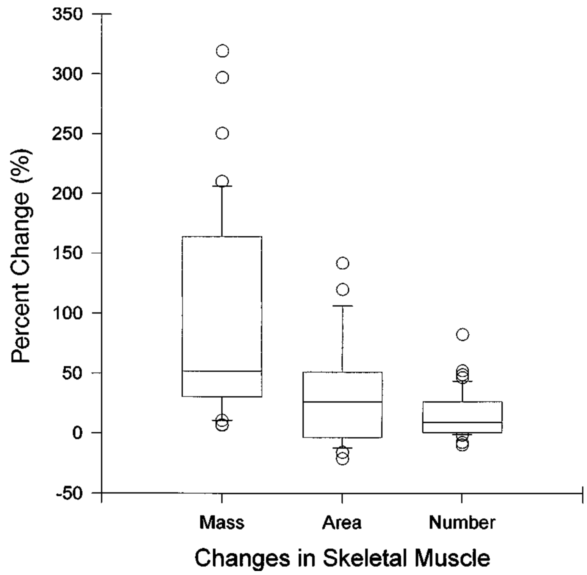

Fig. 1. Percent changes in skeletal muscle mass (n = 37), fiber area (n = 25), and fiber number (n = 37). ○, Outliers beyond 10th and 90th percentiles. Percent change calculated as (treatment − control)/ treatment × 100.

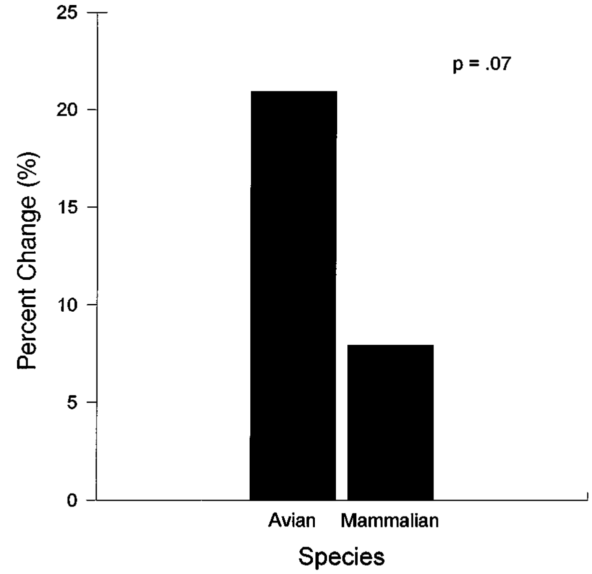

Fig. 3. Percent increases in muscle fiber number according to whether species was avian (n = 20) or mammalian (n = 17). Percent change calculated as (treatment − control)/treatment × 100.

sum test. 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 $P \leq 0.05$.

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

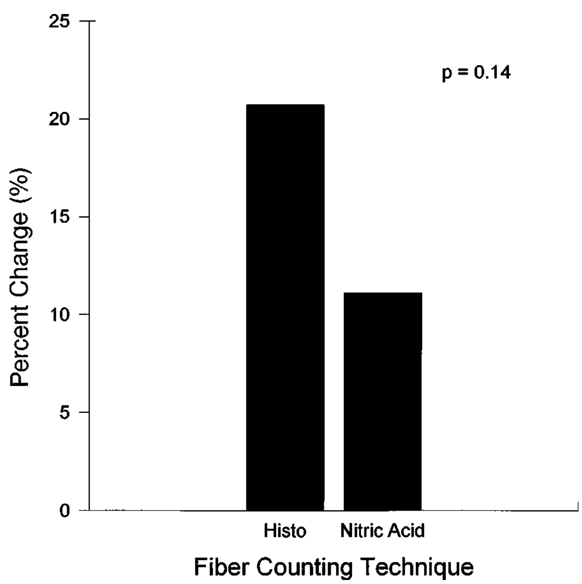

Fig. 2. Percent increases in muscle fiber number according to whether histological (Histo; n = 15) or nitric acid digestion (n = 22) method was used. Percent change calculated as (treatment − control)/ treatment × 100.

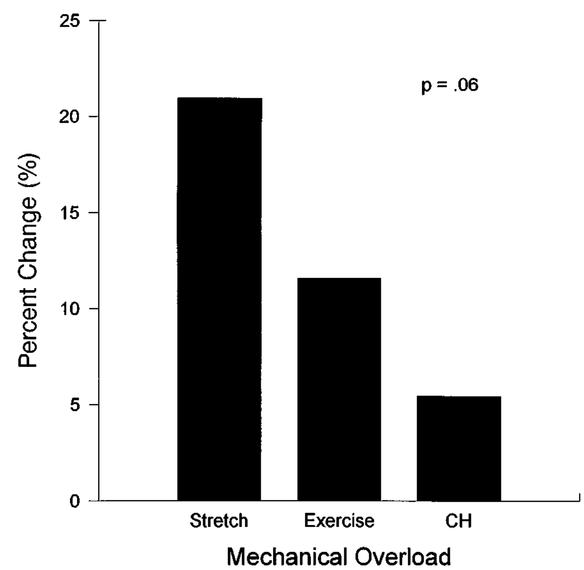

Fig. 4. Percent increases in muscle fiber number according to whether mechanical overload consisted of stretch (n = 20), compensatory hypertrophy (CH; n = 10), or exercise (n= 7). Percent change calculated as (treatment − control)/treatment × 100.

muscle for increased skeletal muscle fiber number. All of the studies used nitric acid digestion and/or histological 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 interval = 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 percent confidence interval = 16.83–46.37) were found (Fig. 1). Examination of outlier groups revealed no physiological reason to exclude them from the analysis. Increases in fiber area were approximately twice as great as increases in muscle fiber number ($P = 0.27$). Changes in muscle mass, fiber area, and fiber number ranged from 6 to 318%, from −21 to 141%, and from −10 to 82%, respectively.

When partitioned according to 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%; Fig. 2). Changes in muscle fiber number categorized according to species examined are found in Fig. 3. Increases in fiber number were greater among those groups that used avian (20.95%) vs. mammalian (7.97%) species. Changes in muscle fiber number partitioned by type of overload are found in Fig. 4. Stretch overload (20.95%) yielded larger increases in muscle fiber number than did exercise (11.59%) and compensatory hypertrophy (5.44%). In addition, no statistically 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%; $P = 0.61$).

DISCUSSION

This meta-analysis attempted to quantify the magnitude 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 (hypertrophy), and muscle fiber number (hyperplasia). Not surprisingly, increases in fiber area were approximately 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 overload 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 established 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 increases 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 mechanical overload employed on all avian species included in this meta-analysis. The fact that increases in fiber number were approximately twice as great when histological 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 compensatory hypertrophy) currently used to examine exercise-induced muscle enlargement truly represents the human 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 species certain forms of mechanical overload increase muscle fiber number.

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