TITLE:

Versatility for Resistance Training and Assessment Using Static and Dynamic Ladders in Animal Models

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SUMMARY:

Resistance training and testing using static and dynamic ladders in animal models

ABSTRACT:
Resistance training is a physical exercise model with profound benefits for health throughout life. The use of resistance exercise animal models is a way to gain insight into the underlying molecular mechanisms that orchestrate these adaptations. The aim of this article is to describe exercise models and training protocols designed for strength training and evaluation of resistance in animal models. In this article, strength training and resistance evaluation are based on ladder climbing activity, using static and dynamic ladders. These devices allow a variety of training models as well as precise control of the main variables which determine resistance exercise: volume, load, velocity, and frequency. Furthermore, unlike resistance exercise in humans, this is forced exercise. Aversive stimuli must be avoided in this intervention to preserve animal welfare. Prior to implementation, a detailed design is necessary, along with an acclimatization and learning period. Acclimatization to training devices, such as ladders, loads, and clinical tape, as well as to the manipulations required, is necessary to avoid exercise rejection and to minimize stress. At the same time, the animals are taught to climb up the ladder, not down, to a safe rest area on the top of the ladder. Resistance evaluation has characterization value of physical strength and permits adjusting and quantifying the training load and the response to training. Furthermore, different types of strength can be evaluated. Regarding training programs, with appropriate design and device use, they can be sufficiently versatile to modulate different types of strength. Furthermore, they should be flexible enough to be modified depending on the adaptive and behavioral response of the animals or the presence of injuries. In conclusion, resistance training and assessment using ladders and weights are versatile methods in animal research.

INTRODUCTION:
Physical exercise is a determinant lifestyle factor for promoting health and decreasing the incidence of the most prevalent chronic diseases as well as some types of cancer in humans [1].

Resistance exercise has raised interest because of its overwhelming relevance for health throughout life[2], especially due to its benefits in counteracting age-related diseases that affect the locomotor system, such as sarcopenia, osteoporosis, etc.[3] Moreover, resistance exercise also affects tissues and organs not directly involved in the execution of movement, such as the brain [4]. This relevance in recent years has encouraged the development of resistance exercise models in animals to study the underlying tissular and molecular mechanisms, when it is not possible in humans or when the animals provide better insight and are a more controlled model.

Unlike resistance exercise in humans, for animal models, researchers usually rely on forced procedures. Aversive stimuli must be avoided in this context mainly to preserve animal welfare, reduce stress, and decrease the severity of the experimental procedures[5]. It should be noted that animals enjoy exercise even in the wild [6]. For these reasons, it is necessary to improve adaptation to the experiment through prolonged stepwise acclimatization.

The devices, materials, and protocols used for resistance training and assessment in experimental animals must allow the precise control and modulation of numerous variables: load, volume, speed, and frequency[7]. They should also allow different types of contractions to be performed:

concentric, eccentric, or isometric. Considering the above, the protocols used should be able to specifically evaluate or train for different applications of strength: maximal strength, hypertrophy, speed, and endurance.

There are several methods of strength training, such as jumping in water[8,9], weighted swimming in water[10], or muscle electrostimulation[11]. However, static and dynamic ladders are versatile devices that are widely used[12,13,4,14].

Resistance assessment in experimental animal models provides valuable information for many research settings, such as describing the phenotypic characteristics of genetically modified animals, evaluating the effect of different intervention protocols (dietary components supplementation, drug treatments, microbiota transplantation, etc.), or assessing the effect of training protocols. Training models provide insight into the physiology of adaptation to strength exercise, that is relevant to health in order to better understand the effect of exercise on health status and pathophysiology.

Consequently, there is no universal protocol for resistance training or the functional assessment of strength in animal models, so versatile protocols are needed.

The aim of this study is to identify the most relevant factors to be considered when designing and applying a protocol for the training and evaluation of resistance using static and dynamic ladders in animal models, providing specific examples.

PROTOCOL:

The methods presented in this protocol have been evaluated and approved by the animal research technical committee (reference PROAE 04/2018, Principado de Asturias, Spain).

1. **Planning**

1.1. Carefully select the animals for study based on the characteristics of interest (genetically modified, pathology models, age, etc.) and apply specific adaptations to the protocol (climbing without weights, reducing inclination, and the number of rungs).

1.2. Identify the strength modality to be assessed or trained: maximal strength, endurance-resistance, speed, etc. depending on the objectives of the study.

1.3. Adjust the parameters carefully when functional assessment or training is framed, considering whether it focuses on the results of these tests or whether they are complementary to other types of clinical, functional, histological, or molecular determinations.

1.4. Plan all issues related to training, particularly the timetable, duration of the training period, and frequency of sessions, and draw a training table.

1.4.1. Specify the warm-up steps and the inclination of the ladder, which will be the same throughout the training. In the training session, the inclination of the ladder is set at 85°. Specify sets, repetitions, load (based on the results of the resistance tests prior to the training period), and rest in between, paying attention to load increases based on the previous session.

1.4.2. Modify the plan, as with human training, depending on the welfare of the animal. Modifications include decreasing repetitions, increasing rest time between sets or repetitions, and decreasing load to avoid overtraining and injury.

1.6. Upon completion, submit the design for evaluation and approval by the animal ethics research committee.

2. **Devices and materials for resistance exercise**

2.1. Static and dynamic ladders

NOTE: Two types of ladders, so-called static and dynamic ladders (See **Figure 1**), can be used for resistance training and evaluation (**Table of materials**).

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Static ladder	in-house production		Figure 1 A
Dynamic ladder	in-house production		Figure 1 B
Weights	in-house production		
Wire for holding weigths	in-house production		
Gator Clip Steel NON-INSUL 10A	Digikey electronics	BC60ANP	
Elastic adhesive bandage 6 cm x 2.5 m	BSN medical	4005556	

2.1.1. Use a vertical ladder with at least 30 steel wire steps of 1.5 mm diameter, separated by 15 mm, and a resting area of at least 20 × 20 cm on the top of the ladder. The slope of the ladder must be adjustable from 80° to 110° with the horizontal plane (**Figure 1C**). Delimit two lanes to prevent non-linear climbing.

2.1.2. Use a dynamic ladder similar to the static ladder (step 2.1.1), with a plastic filament barrier at the top, that can be opened to control access to the resting area, and a plastic filament barrier at the bottom, to prevent the animals from climbing down. The angle of inclination of the ladder must be adjustable between 80° and 100°, the most common being 85°.

NOTE: The ladder has a circular movement by means of an upper and a lower shaft with a diameter of 8 cm. Lower shaft is driven by an electric motor which makes the steps descend at the front and ascend at the rear, creating an endless ladder. It is equipped with a reduction gear and a speed regulator for lowering the speed from 11.6 cm/s to 3.3 cm/s, and the most common speed is 5.6 cm/s.

[Place **Figure 1** here]

2.2. Materials

2.2.1. Prepare the following materials: weights, wire for holding weights, steel gator clip, and clinical adhesive tape.

NOTE: The weights are steel cylinders of different mass (5, 10, 15, 20, 25, and 50 g), with a hole of 5 mm diameter in the center so they can be inserted on a steel wire (**Table of materials**). The wire to hold the weights is made of steel with a diameter of 1-1.5 mm and a length of 5-10 cm, depending on the number of weights to be loaded.

2.2.2. Cut a piece of clinical adhesive tape (**Table of materials**: Elastic adhesive bandage 6 cm x 2.5 m) of approximately 3.0-3.5 x 1.0-1.5 cm size and attach it around the animal's tail to hold the weights. Be sure not to over-tighten as it may lead to blood flow restriction.

NOTE: At first, the animals' behavior is to fight against it and bite the tape, but after a couple of days, they tolerate it, showing no signs of stress and grooming as usual.

2.2.3. Insert the desired weights in the wire and hook the gator clip (**Table of materials**: Steel gator clip and wire for holding weights).

2.2.4. Clamp the gator to the clinical tape attached to the animal's tail.

2.2.5. Immediately after climbing the required rungs, remove the clamp and allow the animal to rest with the clinical tape on the tail, but without the weight (**Figure 1**).

3. **Acclimatization**

NOTE: Proper acclimatization is essential to avoid exercise rejection and to minimize stress. Acclimatization is a crucial stage before resistance evaluation tests or training protocols are performed and adequate time should be spent to achieve behavioral signs of comfort in the animals. Details of daily acclimatization with the static ladder are in **Table 1** and with the dynamic ladder are in **Table 2**.

3.1. Accustom the animals to stay in the resting area at the top of the ladder (static or dynamic). Leave the animals in this place in groups of four, with bedding from their cage, for 15 min every day. Usually, after 3-5 days, the animals show no signs of stress.

3.2. Teach animals to climb up, not down, the ladder. Using the static ladder, place the mice on a rung close to the top of the ladder, from where they can see the resting area, and they will instinctively go to it. Then teach them progressively to climb up from 5 rungs (3x) the first day, to 10 rungs (3x) the following day, up to 15 rungs (3x) (**Table 1**).

3.3. Use the same procedure with the dynamic ladder, first without movement and then, with the ladder moving at 5.4 cm/s and 6.6 cm/s and the animals climbing up for 2 min, completing 5 series (**Table 2**).

3.4. Adapt the animals to carry weights, starting from the third day of acclimatization. Stick a piece of clinical tape to the base of the tail which will be used to hold weights.

3.5. From the seventh day of acclimatization, attach small weights (5-10 g) to the clinical tape with a gator clip. Avoid performing too many series, so the adaptation is not transformed into training.

NOTE: Acclimatization of the control group is mandatory in case this group performs the resistance test. After this period, perform a ladder-climbing reminder once a week.

4. **Protocols for resistance evaluation**

4.1. Incremental tests to assess maximal strength

NOTE: This test intends to determine the maximal resistance measured as the maximum weight at which the animals can climb 10 rungs on the static ladder, which defines the 10-repetition maximum (10RM)[4]. This protocol was adapted from previous studies (reviewed in Kregel et al. [15]).

4.1.1. For warming-up perform 3 series of 10-repetitions, 10 steps/repetition, without external load. For the first series set the slope at 90°, and thereafter at 85°. Allow a rest period of 60 s between series.

4.1.2. Set the slope at 85° (to prevent the weights from grazing or hooking on the rungs of the ladder).

4.1.3. Attach the tape around the animal's tail to hold the weights and prepare the weights as explained in steps 2.2.2-2.2.4.

4.1.4. Start the test with an external load of 10 g and perform one series of 10 steps.

4.1.5. Remove the weight and allow a rest period of 120 s in the resting area.

4.1.6. Perform successive series of 10 steps increasing the external load by 5 g until exhaustion. Allow the resting period (120 s) between series.

4.1.7. If one animal fails to climb 10 steps with a particular weight load, allow for another attempt with the same load after 120 s of rest. If it succeeds to climb with the load, it continues the test with the next load. If it fails again, record the weight load of the last complete series as the maximal weight load.

4.1.8. The test result can be expressed as absolute external weight (g), as maximum load in relation to body weight (%), or as the mass lifted per gram of body weight, as per the discretion of the researcher.

NOTE: The previous protocol[16] represents a model on which numerous modifications are possible, based on the characteristics of the animal model or the animals' status. For example, this protocol has been adapted to assess the maximal resistance of genetically modified mice with neuromuscular disabilities. These animals are not able to climb with external loads and have difficulties climbing 10 rungs with the ladder set at 90° of slope (unpublished data). The adapted protocol consisted of climbing 5 steps without external load, starting with a slope of 110°. The slope increased 5° in each series until 85°. Again, mice rested for 120 s in the resting area after each series. If one animal failed to climb 5 steps at a particular slope, it was allowed another try with the same slope after 120 s of rest. In this case, maximal resistance was expressed as the accumulated number of steps climbed (without considering repetitions after failures). The wild-type control group, after reaching the 85° slope, can continue with the test by adding external weight to the tail, following previous protocol, until exhaustion. Maximal resistance is expressed as the accumulated number of rungs climbed.

4.2. Maximal endurance-resistance test with the static ladder

4.2.1. For warming-up perform 3 series of 10-repetitions, 10 steps/repetition, without external load. For the first series set the slope at 90°, and thereafter at 85°. Allow a rest period of 60 s between series.

4.2.2. Set the slope at 85°.

4.2.3. Clip the weight on the clinical tape placed around the tail of the mouse.

NOTE: Depending on the age and the characteristics of the animals, the external load can be the maximum weight obtained in a previous incremental test, a percentage of it (e.g., 50%), or a percentage of body weight (e.g. 100-200%). If this test is performed after a period of training, it is recommended to use the same load as in the initial test to assess the changes.

4.2.4. Perform consecutive series of 10 steps until exhaustion. No resting time is allowed after each series.

4.2.5. The test result is the number of climbed rungs. If this test is performed before and after a period of training, it is recommended to use the same load.

4.3. Maximal endurance-resistance test with the dynamic ladder

NOTE: The use of the dynamic ladder allows the researcher to control the climbing speed.

4.3.1. Set the slope at 85°.

4.3.2. Set the speed at 4.2 cm/s.

4.3.3. For warming-up perform 3 series of 100 steps, without external load. Allow a rest period of 60 s between series.

4.3.4. Clip the weight on the clinical tape placed around the tail of the mouse.

NOTE: Depending on the age and the characteristics of the animals, the external load can be the maximum weight obtained in a previous incremental test, a percentage of it (e.g., 50%), or a percentage of body weight (e.g. 100-200%). If this test is performed after a period of training, it is recommended to use the same load as in the initial test to assess the changes.

4.3.5. Start at 4.2 cm/s and the speed is increased by 1.2 cm/s every 60 s until exhaustion.

4.3.6. The test result is the exercise time, the number of climbed rungs, or the maximum speed. If this test is performed before and after a period of training, it is recommended to use the same load.

5. **Protocol for resistance training with static ladder**

NOTE: Before starting the training period, acclimatization (**Table 1)** and training planning are necessary. To reduce anxiety, adapt and train the mice in groups of 4 animals sharing the same cage.

5.1. For warming-up perform 3 series of 10 repetitions, 10 steps/repetition, without external load. For the first series set the slope at 90°, and thereafter at 85°. Allow a rest period of 60 s between series.

5.2. Training session starts in the resting area. Clip the gator with the weight on the clinical tape.

5.3. Gently place the mouse 10-20 rungs below the resting place. Allow the mouse to grip the rung and climb to the resting area.

5.4. Repeat this process (5.3.) until the number of rungs in this series (e.g.,10 rungs x 10 series) is completed.

5.5. Remove the weight from the mouse tail and wait for 120 s until the next series.

5.6. Increase the number of steps and the maximum weight loads of the series throughout the training period, while maintaining the weekly schedule.

NOTE: An example of the variation of loads during a week planning is shown in **Table 3**. Shortly, Tuesday and Friday with high weight load (40-50 g) and a low number of steps (500-400); Monday and Thursday with intermediate weight load (25-35 g) and an intermediate number of steps (800-600); and Wednesday without weight load but a high number of steps (2000). This design is intended to facilitate recovery from previous training sessions and avoid injuries and overtraining. Since multiple designs are possible, **Table 4** shows examples of three weeks of training (at the beginning, in the middle, and at the end of the training period, respectively)[4]. The static ladder is also suitable for eccentric training. It can be performed by descending with a near-maximal or supramaximal load. The load applied for this procedure must be high (e.g., 90-100% or even more of the maximum incremental concentric test). When mice carry a near-maximal load, they naturally try to descend. In the case of eccentric training, during acclimatization, it is necessary to allow the animals to descend rather than ascend. For this reason, it is not easy to combine both concentric and eccentric training in mice, and only one training model is feasible at a given time.

6. **Protocol for resistance training with dynamic ladder**

NOTE: After acclimatization, the training on the dynamic ladder is quite like the static one (**Table 2**). Training is performed on 2-4 mice at a time.

6.1. Set the slope to 85°, close the door to the resting area, and start the ladder at the desired speed (e.g., 5.4 cm/s).

6.2. For warming-up perform 3 series of 100 steps, without external load. Allow a rest period of 60 s between series.

6.3. When the mouse is in the resting area, clip the gator with the weight on the clinical tape. Alternatively, the weight can be attached when the mouse is on the ladder.

6.4. Gently place the mouse at the top of the moving staircase with the weight on the tail. Allow the mice to grip the rung and climb.

6.5. When the number of rungs in this series is reached (e.g., 100), the weights are removed, and the door is opened so that the animal can go to the resting area. The rest time is 120 s before the next series.

NOTE: The number of steps climbed is counted as a function of the climbing time at the set speed.

6.6. This procedure is repeated until the training session is completed. The detailed daily training program is shown in **Table 5**.

7. **Evaluation of the crossover effect of resistance training on endurance performance**

NOTE: For this, an incremental treadmill test is performed[4], after 24 h of rest.

7.1. After a warm-up of 3 min at 10 cm/s, start the incremental test at 10 cm/s and 10° angle of inclination.

7.2. Increase the speed by 3.33 cm/s every 3 min until exhaustion.

NOTE: No electric shocks are used, so a painter's brush is placed at the back of the treadmill to prevent the mice from running off it.

8. **Animal behavior during procedures**

NOTE: Continuous monitoring of the adaptation of mice to training should be performed to detect extreme fatigue, overtraining, or injury.

8.1. Observe signs of animal welfare, in particular grooming and refusal to training. The normal behavior of the mouse, after a series of intense training, is to remain inactive for about one minute due to fatigue. After that, they start grooming, exploring, or trying to remove the tape on the tail.

8.2. In the case of a mouse refusing to train a series, try giving longer rests or even not performing the series to prevent inhibition.

8.3. Occasionally, when carrying out lightweight exercises, gently push the animal's tail, to encourage it to finish the series. The animals stop climbing because it is not a demanding task. Conversely, when animals are carrying a heavy load, gently shift the animal's weight to ease the load and encourage it to finish the series, and then allow the animal to rest until the next training session. The animals may stop or even attempt to descend because of the heavy load

9. **Safety procedures**

9.1. Security procedures for researchers- Conduct research in the animal facility laboratory and use hoods, hats, gloves, and masks. There are no additional requirements other than those specific to animal research.

9.2. Security for animals- During the test, place a hand under the weights to catch and hold the mice in case of exhaustion because the animal is limited in its ability to hold on to the rungs properly. Pay attention to the animals continuously for potential risks during training such as falls or jumps.

REPRESENTATIVE RESULTS:

Results with static ladder

The progressive resistance training protocol used in the study of Codina-Martinez et al. [4] (Table 4) was tested in a preliminary study consisting of 7 weeks of training on a static ladder with 6-months-old wild-type C57BL6J mice (n=4). In this preliminary study, incremental tests to assess maximal strength were performed before and after the training period. We observed a 46.4 % increase in maximal strength, meaning that at the end of the training period they were able to climb with 1.9 times their body weight (unpublished data).

In the study of Codina-Martínez et al.[4], male mice (C57BL6/129Sv) deficient in *Atg4b* [16] and their corresponding wild-type controls (8 weeks old) were trained for 14 weeks (Table 4). Incremental tests to assess maximal resistance, before and after the training period, showed a percentage change of 44% in trained wild-type animals and 15.3% in *atg4b*$^{-/-}$mice.

In another study of our research group, 8-week-old C57BL6N mice were trained for 4 weeks, 5 days/week (n=8) (unpublished data). All sessions were designed to achieve the same exercise volume through a combination of the number of steps climbed (or distance against gravity) and weight load [17] and based on the results obtained in a maximal strength test prior to the training period. The number of steps per training session varied between 400-2000 depending on the maximal weight load, which ranged between 25-65% of the maximal weight load at the pre-training test. We selected these maximum weight ranges because it has been described that below 75% of 1RM there is no velocity loss, which is important for standardizing the intensity of submaximal efforts[18]. Again, before and after the training period, incremental tests to assess maximal strength were performed. The average percentage of variation in this parameter was 40%. Peak strength was reached by a 27 g mouse, which was able to climb 1RM with 120 g after the training period.

Results with dynamic ladder

To evaluate the dynamic ladder as a tool for resistance training, we conducted an experiment with the aim of assessing the effect of two types of strength training: endurance-resistance training and strength training. 8-week-old C57BL6N mice were divided into three groups: Non-trained control (C, n=5), Endurance-Resistance (E-R, n=8), and Strength (S, n=7). After a 3-weeks (12 sessions) acclimatization period (**Table 2**) mice were trained for 6 weeks, 5 days/week (Monday to Friday), starting at 9:00 am, for a total of 22 sessions. To reduce anxiety, mice were trained in groups of 4 animals sharing the same cage. Aversive stimuli were also avoided, to minimize stress. The E-R group performed 3 times more repetitions with 1/3 of the weight load compared to the S group, so, they all performed the same accumulated work, with different combinations of load and repetitions. The speed was constant for all groups, set at 5.4 cm/s and the slope at 85°.

The normality of the variables was tested using the Shapiro Wilk test. Results are shown as mean ± SD. t-test and ANOVA (Bonferroni post-hoc) were used for statistical differences. Significant changes are set at $p<0.05$. The statistical software R (www.r-project.org) was used for all statistical analyses.

All animals included in the trained and control group completed the study. During the experiment, the animals were caged in training groups. The food intake of the animals in each cage was measured weekly, so the result is per cage and not per mouse. The mean daily food intake per mouse in the control group was 2.8(± 0.11) g, in the Endurance-Resistance group was 3.2(± 0.24) g, and in the strength group was 3.3(± 0.13) g. Exercised mice had a higher food intake than controls ($p<0.05$). However, there was no difference in body weight after the intervention (C group 28.0 ± 3.18 g, E-R group 28.5 ± 1.93, and S group 28.1 ± 2.52 g).

The effect of a period of 6 weeks of strength training in a dynamic ladder on the resistance is shown in **Figure 2 and Figure 3**. Two models of strength training were conducted: endurance-resistance training and strength training. The variation in maximal strength (**Figure 2**) shows a significant increase after training in S (29.5 ±1 0.9 % increase, p=0.021) and E-R groups (41.5 ± 2.5 % increase, p=0.0004), while this parameter did not change in C (20.0 ± 4.0 %, p=0.201). Endurance-resistance measured at the end of the training period (**Figure 3**) was significantly higher in the E-R group as compared to S (122.5 vs 26.9 rungs, p=0.005) and C groups (122.5 vs 18.8 rungs, p=0.013).

The cross-training effect of these models, the effect of strength training on endurance, was also studied. For that purpose, all animals performed incremental maximal endurance tests on a treadmill before and after the training period, according to the protocols previously described [19]. A significant loss in endurance was observed in C (Pre: 1219 ± 133 s vs. Post: 982 ± 149 s, p=0.004), while no significant changes were observed for S (Pre: 1364 ± 285 s vs. Post: 1225 ± 94 s, p=0.253) and E-R (Pre: 1139 ± 96 s vs. Post: 1185 ± 84 s, p=0.164).

FIGURE AND TABLE LEGENDS:

Figure 1. Resistance **training devices: static and dynamic ladders**. (A) Mouse training with external weight in a static ladder; (B) Two mice training with weight in a dynamic ladder; (C) Schematic representation of ladder angles for training.

Figure 1

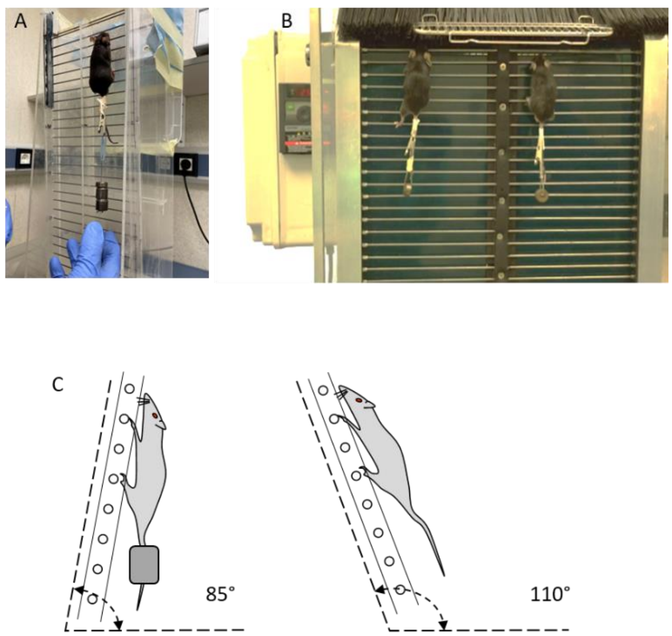

Figure 2. Maximal strength, before and after a 6-week resistance training period in a dynamic ladder, measured using an incremental test. Legend: * p<0.05; ** p<0.01.

Figure 2

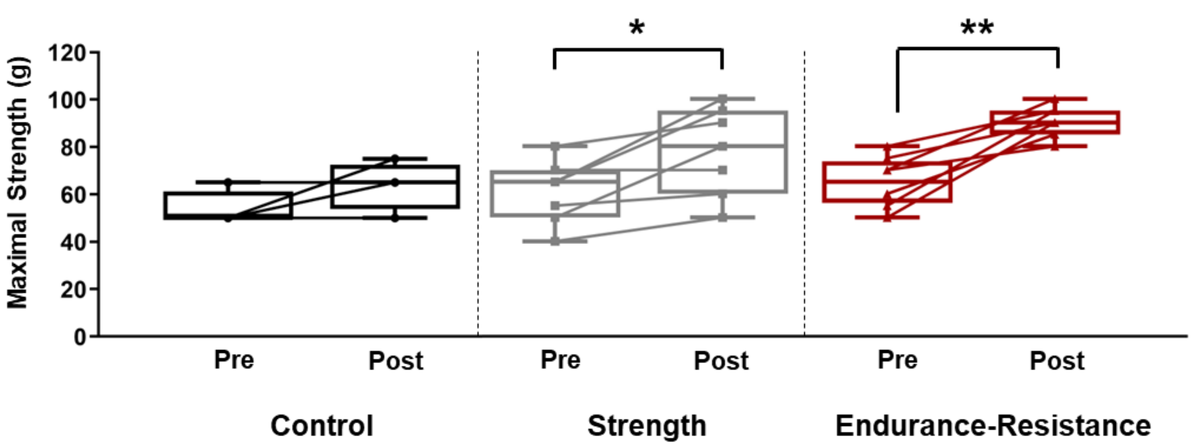

Figure 3. Maximal endurance-resistance, before and after a 6-week resistance training period in a dynamic ladder, using an maximal endurance-resistance test. Legend: C: Control; S: Strength and E-R: Endurance-Resistance. * p<0.05.

Figure 3

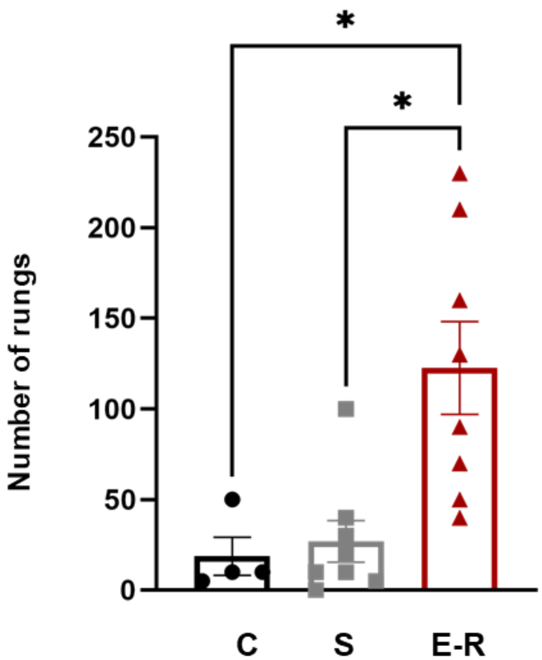

Table 1. Example of a 10-day acclimatization protocol in a static ladder with wild-type mice.

	Static Ladder				
Day	Time (min)	Rungs (n)	Inclination (°)	Tape	Weight (g)
1	15	0	90		
2	15	0	90		
3	15	5 (x3)	90		
4	15	5 (x3)	90	Tape	
5	15	10 (x3)	90	Tape	
Rest					
Rest					
6	15	10 (x5)	85	Tape	
7	15	10 (x5)	85	Tape	5
8	15	10 (x5)	85	Tape	5
9	15	10 (x5)	85	Tape	5
10	15	10 (x5)	85	Tape	5

Table 2. Example of a 13-day acclimatization protocol in a static ladder with wild-type mice.

	Dynamic ladder						
Day	Time (min)	Speed cm/s	Series (n)	Rungs (n)	Total rungs (n)	Inclination (°)	Tape/ Weigth (g)
1	15	0	0	0	0	90	0
2	15	0	0	0	0	90	0
3	15	0	1	5	5	90	0
4	15	0	1	5	5	90	0
5	15	0	1	5	5	90	0
Rest							
Rest							
6	15	0	5	10	50	90	0
7	15	0	5	10	50	90	0
	Rest						
8	1	6,65-11,6	1		235	90	0
Rest							
Rest							
Rest							
9	3	5,4	6		5832	85	0
10	3	5,4	6		5832	85	0
11	3	5,4	6		5832	85	Tape
12	3	5,4	2		3540	85	Tape
	1	6,65	4			85	10
13	3	6,65	3		6783	85	Tape
	2	6,65	4			85	10

Table 3. Example of a training week with the static ladder. Legend: Rep: repetitions, Steps: number of rungs climbed, Slope: angle with the horizontal plane, and load: grams attached to the tail.

	Series	Parameters	Units	Mon	Tue	Wed	Thu	Fri
Warm-up	1	Rep	n	10	10	10	10	10
		Steps	n	10	10	10	10	10
		slope	°	90	90	90	90	90
		Load	g	0	0	0	0	0
	2	Rep	n	10	10	10	10	10
		Steps	n	10	10	10	10	10
		slope	°	85	85	85	85	85
		Load	g	0	0	0	0	0
	3	Rep	n	10	10	10	10	10
		Steps	n	10	10	10	10	10
		slope	°	85	85	85	85	85
		Load	g	0	0	0	0	0
Training	4	Rep	n	10	10	10	10	10
		Steps	n	10	10	25	10	10
		slope	°	85	85	85	85	85
		Load	g	25	40	0	35	50
	5	Rep	n	10	10	10	10	10
		Steps	n	10	10	25	10	10
		slope	°	85	85	85	85	85
		Load	g	25	40	0	35	50
	6	Rep	n	10	10	10	10	10
		Steps	n	10	10	25	10	10
		slope	°	85	85	85	85	85
		Load	g	25	40	0	35	50
	7	Rep	n	10	10	10	10	10
		Steps	n	10	10	25	10	10
		slope	°	85	85	85	85	85
		Load	g	25	40	0	35	50
	8	Rep	n	10	10	10	10	
		Steps	n	10	10	25	10	
		slope	°	85	85	85	85	
		Load	g	25	40	0	35	
	9	Rep	n	10		10	10	
		Steps	n	10		25	10	
		slope	°	85		85	85	
		Load	g	25		0	35	
	10	Rep	n	10		10		
		Steps	n	10		25		
		slope	°	85		85		
		Load	g	25		0		
	11	Rep	n	10		10		
		Steps	n	10		25		
		slope	°	85		85		
		Load	g	25		0		
		Accumulated						
		Steps	n	800	500	2000	600	400
		Altitude	cm	1200	750	3000	900	600

Table 4. Example of three weeks of training with the static ladder. Labeled as low (sessions 1-4), medium (10-14), and high load (30-34). Legend: Rep: repetitions, Steps: number of rungs climbed, Slope: angle with the horizontal plane, and load: grams attached to the tail. This table is adapted from Codina-Martinez et al. 2020[4].

			Low				Medium					High				
	Parameters	Units	Mon	Tue	Thu	Fri	Mon	Tue	Wed	Thu	Fri	Mon	Tue	Wed	Thu	Fri
Series																
1	Rep	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Steps	n	15	15	15	15	15	15	15	15	15	15	15	15	15	15
	slope	°	90	90	90	90	90	90	90	90	90	90	90	90	90	90
	Load	g	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Rep	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Steps	n	15	15	15	15	15	15	15	15	15	15	15	15	15	15
	slope	°	85	85	85	85	85	85	85	85	85	85	85	85	85	85
	Load	g	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Rep	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Steps	n	15	15	15	15	15	15	15	15	15	15	15	15	15	15
	slope	°	80	80	80	80	g	80	80	80	80	80	80	80	80	80
	Load	g	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Rep	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Steps	n	15	15	15	10	10	15	10	15	10	15	15	15	15	15
	slope	°	80	80	80	85	85	80	85	80	85	85	85	85	85	85
	Load	g	0	0	0	15	25	0	25	0	25	10	0	10	0	10
5	Rep	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Steps	n	15	10	15	10	10	15	10	15	10	15	15	15	15	15
	slope	°	80	85	80	85	85	80	85	80	85	85	85	85	85	85
	Load	g	0	15	0	15	25	0	25	0	25	10	0	10	0	10
6	Rep	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Steps	n	15	10	15	10	10	15	10	15	10	10	15	10	15	10
	slope	°	80	85	80	85	85	80	85	80	85	85	85	85	85	85
	Load	g	0	15	0	15	25	0	25	0	25	25	0	30	0	30
7	Rep	n			10		10	10	10	10	10	10	10	10	10	10
	Steps	n			15		10	15	10	15	10	10	15	10	15	10
	slope	°			80		85	80	85	80	85	85	85	85	85	85
	Load	g			0		25	0	25	0	25	25	0	30	0	30
8	Rep	n						10	10	10	10	10		10		10
	Steps	n						15	10	15	10	10		10		10
	slope	°						80	85	80	85	85		85		85
	Load	g						0	25	0	25	25		30		30
9	Rep	n									10	10		10		10
	Steps	n									10	10		10		10
	slope	°									85	85		85		85
	Load	g									25	25		30		30
10	Rep	n										10				10
	Steps	n										10				10
	slope	°										85				85
	Load	g										25				30
11	Rep	n										10				10
	Steps	n										10				10
	slope	°										85				85
	Load	g										25				30
	Accumulated															
	Steps	n	900	800	1050	750	850	1200	950	1200	1050	2100	1050	1150	1050	2100
	Altitude	cm	1350	1200	1575	1125	1275	1800	1425	1800	1575	3150	1575	1725	1575	3150

Table 5. Example of training with dynamic ladder. Program of two groups of endurance-resistance and strength training. Legend: The warm-up is common to both groups. The slope is set at 85°.

	WARM UP				ENDURANCE-RESISTANCE			RESISTANCE		
day	Series (n)	time (min)	Speed (cm/s)	Weight (g)	Series (n)	Time (min)	Weight (g)	Series (n)	Time (min)	Weight (g)
1	3	3	5,4	0	6	3	3	6	1	10
2	3	3	5,4	0	6	3	3	6	1	10
3	3	3	5,4	0	6	2	0	6	2	0
4	3	3	5,4	0	5	3	5	5	1	15
5	3	3	5,4	0	5	3	5	5	1	15
6										
7										
8	3	3	5,4	0	5	3	5	5	1	15
9	3	3	5,4	0	6	3	5	6	1	15
10	3	3	5,4	0	6	2	0	6	2	0
11	3	3	5,4	0	6	3	5	6	1	15
12	3	3	5,4	0	6	3	5	6	1	15
13										
14										
15	3	3	5,4	0	6	3	5	6	1	15
16	3	3	5,4	0	6	3	7	6	1	20
17	3	3	5,4	0	6	2	0	6	2	0
18	3	3	5,4	0	6	3	7	6	1	20
19	3	3	5,4	0	6	3	7	6	1	20
20										
21										
22-25	Behaviour test									
26	3	3	5,4	0	4	3	5	4	1	15
27										
28										
29	6	3	5,4	0	6	3	5	6	1	15
30	3	6	5,4	0	6	3	7	6	1	20
31	TEST									
32	3	6	5,4	0	4	3	5	4	1	15
33	TEST									
34										
35										
36	3	6	5,4	0	6	3	7	6	1	20
37	6	3	5,4	0	6	3	7	6	1	20
38	6	3	5,4	0	6	2	0	6	2	0
39	TEST									
40	6	3	5,4	0	5	3	7	5	1	20
41										
42										
43	3	6	5,4	0	6	3	7	6	1	20
	SACRIFICE									

DISCUSSION:

Training is an intervention with multiple applications in research, apart from the study of exercise itself[20]. Thus, the analysis of its effect on ageing[21] or certain pathological conditions and physical therapy[22] has received much attention in recent years. In addition, numerous authors have analyzed the effect of pharmacological[23] or dietary[22] interventions on physical fitness. In this context, interest has arisen in analyzing different exercise modalities separately, with an emerging interest in resistance exercise. Resistance exercise elicits a different molecular response to endurance in numerous tissues[24] and has also been shown to have a specific effect on a number of pathological conditions[22].

The use of animal models for the study of resistance exercise is a tool with multiple applications. It allows the characterization of a specific phenotype in models of pathologies or genetically modified animals, although this description is not usually included. In addition, the implementation of exercise protocols and the evaluation of their impact on these models provides insight into the physiology or pathophysiology of these conditions[25].

Some authors have previously conducted resistance training with rats[12,13] and mice[4,14], using different training models. Some authors have applied isometric protocols to train and assess strength[26]. Overload jumping in the water and weighted swimming were also applied [9,10]. Nerve stimulation performed under anesthesia,[11] and combining resistance training with surgical procedures to cause biomechanical muscle overload and muscle hypertrophy[27] have also been done.

However, some of the interventions to improve resistance have some weaknesses. Forced exercise with electric shocks has been shown to interfere with experimental results [28]. Some of the procedures are stressful because they rely on forced swimming to prevent the animal from drowning[9,10]. Nerve stimulation is not a volitional muscle contraction and is performed under anesthesia[11]. The simplest approach to resistance training and assessment is that of non-invasive procedures using concentric/eccentric muscle contractions.

Although the most common devices to apply these protocols are static ladders on which the animals climb with external weights, resistance exercise could also be carried out using dynamic devices. In this regard, Konhilas et al.[29] used weighted wheels. However, this approach is more like a high-intensity endurance exercise, so specificity would be lost. In this article, we show, for the first time, protocols for resistance training and resistance evaluation using a dynamic ladder, which allows for very versatile approaches, as well as the results upon their implementation. In addition, the use of a dynamic ladder means less manipulation of the animals, as they can climb with weight continuously, without the need of climbing a series of steps as with a static ladder.

Force assessment of peak forces can be performed using grip strength[30] and torque generated by direct nerve stimulation[31]. The assessment of strength using the ladders is useful for subsequent training planning. The dynamic ladder also allows time-limit tests to be carried out, evaluating the number of steps as a function of the load. This procedure is equivalent to the maximum number of weight repetitions test performed in humans[7].

Furthermore, in relation to training and assessment methods, our study emphasizes acclimatization as a key factor in avoiding training refusal on both static and dynamic ladders. This acclimatization is not achieved by food reward, as described in Yarsheski et al.[13], but by teaching the mice to reach the resting areas at the top of the ladders, so that they are motivated to climb, without the need for food restrictions. Our goal has been to achieve "humanized animal exercise", as suggested by Seo et al.[32]. In this regard, it is also worth noting that, following this protocol, the mice are trained in a group while maintaining social interaction, unlike other types of training (such as treadmill training) in which the animals are alone. In the protocols shown in this paper, the animals' refusal of training was non-existent in both the static and dynamic ladders, this could be due to the adaptation protocol.

Our results show that different protocols with different animal models were effective in improving maximal strength. They were also sensitive enough to detect differences between genetically modified animals with alterations in muscle function and wild-type animals, both in maximal resistance and in response to training[4]. Furthermore, a comparison of the training programs with the dynamic ladder (C, S, and E-R) showed that all groups of mice increased their maximal strength. For C, this could be because the mice were young and still growing. Even so, the improvement in the S and E-R groups was much greater, which is evidence of the effect of training. Furthermore, in the post-training strength-endurance test, which consisted of climbing as many steps as possible with the maximum weight obtained in the incremental test before training, the E-R group was superior to the S and C groups, which had no significant differences. Furthermore, the incremental treadmill test showed that there was no decrease in endurance in any of the trained groups while a decrease was observed in the C group. This is consistent with the cross-training effect of resistance training on endurance previously described [33]. These results suggest, on one hand, the specificity of the resistance training protocols presented in this study for increasing resistance and endurance capacities. At the same time, both training modalities show a diverse effect on physical fitness[34], probably due to a diverse set of molecular mechanisms triggered by each training model[24].

Finally, although these training models affected the resistance, we have also observed a great heterogeneity both in the starting resistance of the individuals and in the response to training (**Figures 2,3**). This observation is in line with what has been described by other authors[35]. This should be considered when interpreting the results of the intervention in the different parameters to be evaluated in the samples obtained from these animals.

Limitations.
Evaluation of some type of strength such as maximal (isometric) strength is not possible with this protocol so other protocols and devices, such as grip strength, must be used.

Conclusion.
Resistance training and assessment, with a static and dynamic ladder, is a feasible method in animal research with a wide range of protocols depending on the objective of the study.

ACKNOWLEDGMENTS:
This work was supported in part by the Ministerio de Economía y Competitividad, Spain (DEP2012-39262 to EI-G and DEP2015-69980-P to BF-G). Thanks to Frank Mcleod Henderson Higgins from McLeod´s English Centre in Asturias, Spain, for language assistance.

DISCLOSURES:
The corresponding author ensures that all authors have no conflict of interest.

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