

Acute and delayed response to resistance exercise leading or not leading to muscle failure

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Summary

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This study compared the time course of recovery following two resistance exercise protocols differing in the number of repetitions per set with regard to the maximum possible (to failure) number. Ten men performed three sets of 6 versus 12 repetitions with their 70% 1RM (3×6 [12] versus 3×12 [12]) in the bench press (BP) and squat (SQ) exercises. Mechanical [CMJ height, velocity against the 1 m s^{-1} load (V_1 -load)], biochemical [testosterone, cortisol, growth hormone, prolactin, insulin-like growth factor-1, creatine kinase (CK)] and heart rate variability (HRV) and complexity (HRC) were assessed pre-, postexercise (Post) and at 6, 24 and 48 h-Post. Compared with 3×6 [12], the 3×12 [12] protocol resulted in significantly: higher repetition velocity loss within each set (BP: 65% versus 26%; SQ: 44% versus 20%); reduced V_1 -load until 24 h-Post (BP) and 6 h-Post (SQ); decreased CMJ height up to 48 h-Post; greater increases in cortisol (Post), prolactin (Post, 48 h-Post) and CK (48 h-Post); and reductions in HRV and HRC at Post. This study shows that the mechanical, neuroendocrine and autonomic cardiovascular response is markedly different when manipulating the number of repetitions per set. Halving the number of repetitions in relation to the maximum number that can be completed serves to minimize fatigue and speed up recovery following resistance training.

Introduction

Manipulation of the acute resistance exercise variables (i.e. load, number of sets and repetitions, exercise type and order, rest duration and movement velocity) configures the exercise stimulus and determines the magnitude and type of the physiological responses and adaptations consequent to resistance training (RT) (Spiering et al., 2008). One of the key physiological systems in tissue remodelling is the neuroendocrine system (Crewther et al., 2005; Ratamess et al., 2005). Exercise protocols using moderate to high loads (10RM), high volume (3 sets of 10 repetitions per exercise), short inter-set rests (1 min) and stressing a large muscle mass tend to produce the highest elevations in hormones such as testosterone, growth hormone (GH) and cortisol (Kraemer et al., 1990, 1991). Muscle damage induced by such protocols is thought to play a role in muscle hypertrophy (Schoenfeld, 2010). Thus, the greater magnitude and duration of the hormonal response and muscle damage associated with RT to failure would result in an increased muscle hypertrophy (Ahtiainen et al., 2003). In contrast, other studies seem to indicate that exercising to failure may not be a critical stimulus for

strength gains to occur (Folland et al., 2002; Izquierdo et al., 2006; Izquierdo-Gabarren et al., 2010; Sampson & Groeller, 2015).

It has been shown that the mechanical stress and metabolic stress clearly differ when manipulating the actual number of repetitions performed in each training set in relation to the maximum, to failure, number that can be completed (Sánchez-Medina & González-Badillo, 2011). However, to our knowledge, the neuroendocrine response to RT protocols differing in the number of repetitions, i.e. going to muscle failure versus ending each set several repetitions short of failure, has not yet been addressed. In addition, it has been suggested that training to failure may require longer recovery times (Sánchez-Medina & González-Badillo, 2011; Gorostiaga et al., 2012, 2014), which is an important factor for most competitive athletes, as an excessive fatigue resulting from RT might interfere with the development of other physical, technical or tactical components (Draganidis et al., 2013).

Heart rate variability (HRV), analysed using linear time and frequency domain measures (Akselrod et al., 1981), has been used to study the responses of the physiological control system to physical activity (Heffernan et al., 2007, 2008;

Iglesias-Soler et al., 2015; Kingsley et al., 2014). As physiological control mechanisms under healthy conditions exhibit complex dynamics (Costa et al., 2002), a loss of information regarding cardiac autonomic fluctuations might happen if only linear methods are used. Methods derived from the theory of non-linear dynamics, such as multiscale entropy, may provide complementary information about the structure of physiological output signals (Costa et al., 2002). Complexity relates to the irregularity of a dynamic process, which can be estimated by assessing the uncertainty of patterns reoccurring within a time event series (Lipsitz, 1995). Recent studies have compared the effect of training with two different relative loads on cardiac autonomic modulation (Lima et al., 2011; De Souza et al., 2013). A decreased HRV following RT was observed, without significant differences between the loads used. To our knowledge, no study has analysed the effect of two different resistance exercise protocols (REP) leading or not leading to muscle failure using the same relative load (percentage of one-repetition maximum, %1RM) on HRV and heart rate complexity (HRC). It seems worthy of attention to further explore this issue, given the likely greater accuracy of methods derived from the theory of non-linear dynamics.

The disturbance in homeostasis induced by exercise requires coordinated adjustments in the neuroendocrine and autonomic nervous systems (Borresen & Lambert, 2008). The hypothalamus plays a key role in the integration and control of these systems (Kuipers, 1996). As greater mechanical stress and metabolic stress have been observed as the number of repetitions in an exercise set approaches failure (Sánchez-Medina & González-Badillo, 2011), a greater hormonal and cardiac autonomic response would be expected when RT is performed to muscle failure. Therefore, the aim of this study was to analyse the time course of recovery following two different REP in terms of the level of effort required: maximal (to failure) versus half-maximal number of repetitions per set in the fundamental RT exercises of bench press (BP) and full squat (SQ). Several assessment time points up to 48 h postexercise were established to evaluate the mechanical, biochemical and autonomic cardiovascular response to an acute REP in an attempt to advance in our understanding of the overall recovery status following RT.

Methods

Participants

Ten men (age 23.6 ± 3.7 years, height 1.75 ± 0.03 m, body mass 75.0 ± 8.7 kg) volunteered to participate in this study. Subjects were physically active sports science students with a RT experience ranging from 2 to 4 years (1–3 sessions per week). 1RM strength was 108.3 ± 15.0 kg for the SQ and 87.6 ± 20.5 kg for the BP exercise. After being informed about the purpose, testing procedures and potential risks of the investigation, subjects gave their written consent to partic-

ipate. No physical limitations, health problems or musculoskeletal injuries were found after a medical examination. None of the subjects was taking drugs, medications or dietary supplements known to influence physical performance. The study was approved by the Research Ethics Committee of Pablo de Olavide University and was conducted in accordance with the Declaration of Helsinki.

Study design

Following familiarization and initial strength assessment, subjects undertook two randomized REP performed 14 days apart in separate trials. The same relative load (70% 1RM), number of exercise sets (3) and inter-set rest duration (5 min) were used in both REP. The protocols only differed in the actual number of repetitions (R) performed in each set (S) in relation to the maximum possible number of repetitions (P) ($S \times R [P]$: $3 \times 12 [12]$ versus $3 \times 6 [12]$); i.e. the first protocol demanded a maximal effort (to failure, equivalent to $3 \times 12RM$), whereas in the second one, only half the maximum number of repetitions were performed in each set.

To compare the mechanical, biochemical and HRV response, as well as the time course of recovery following the two REP analysed, subjects underwent a battery of measurements at different time points: preexercise (Pre), postexercise (Post), 6 h-Post, 24 h-Post and 48 h-Post (Fig. 1a). We chose to study recovery up to 48 h following each REP because this is a temporal segment usually employed as maximal separation between training sessions, or between a training session and competition in many sports disciplines. Vertical countermovement jump (CMJ) height and the individual load (kg) that elicits a $\sim 1.00 \text{ m s}^{-1}$ (V_1 -load) barbell mean propulsive velocity (from here on, velocity) were assessed at Pre, Post, 6 h-Post, 24 h-Post and 48 h-Post. These mechanical measurements have been described in previous research (Sánchez-Medina & González-Badillo, 2011; Pareja-Blanco et al., 2014). The V_1 -load was chosen because it is a sufficiently high velocity, which is attained against medium loads ($\sim 47\%$ 1RM in BP and $\sim 60\%$ 1RM in SQ) (González-Badillo & Sánchez-Medina, 2010; Sánchez-Medina & González-Badillo, 2011; Pallarés et al., 2014; Pareja-Blanco et al., 2014), and it allows a good expression of the effect of fatigue on velocity, besides being relatively easy to move and quick to determine as part of the warm-up (Sánchez-Medina & González-Badillo, 2011). Blood sampling for the determination of testosterone, cortisol, GH, prolactin (PRL), insulin-like growth factor-1 (IGF-1) and creatine kinase (CK) concentrations was performed at Pre, Post and 48 h-Post. HRV measurements are described later in detail.

Participants abstained from any strenuous physical activity for at least 4 days before each trial. The two REP were performed at the same time of day for each subject and under similar environmental conditions ($20\text{--}22^\circ\text{C}$ and $55\text{--}65\%$ humidity). Subjects underwent four familiarization sessions 2 weeks before the start of the first trial. These sessions were

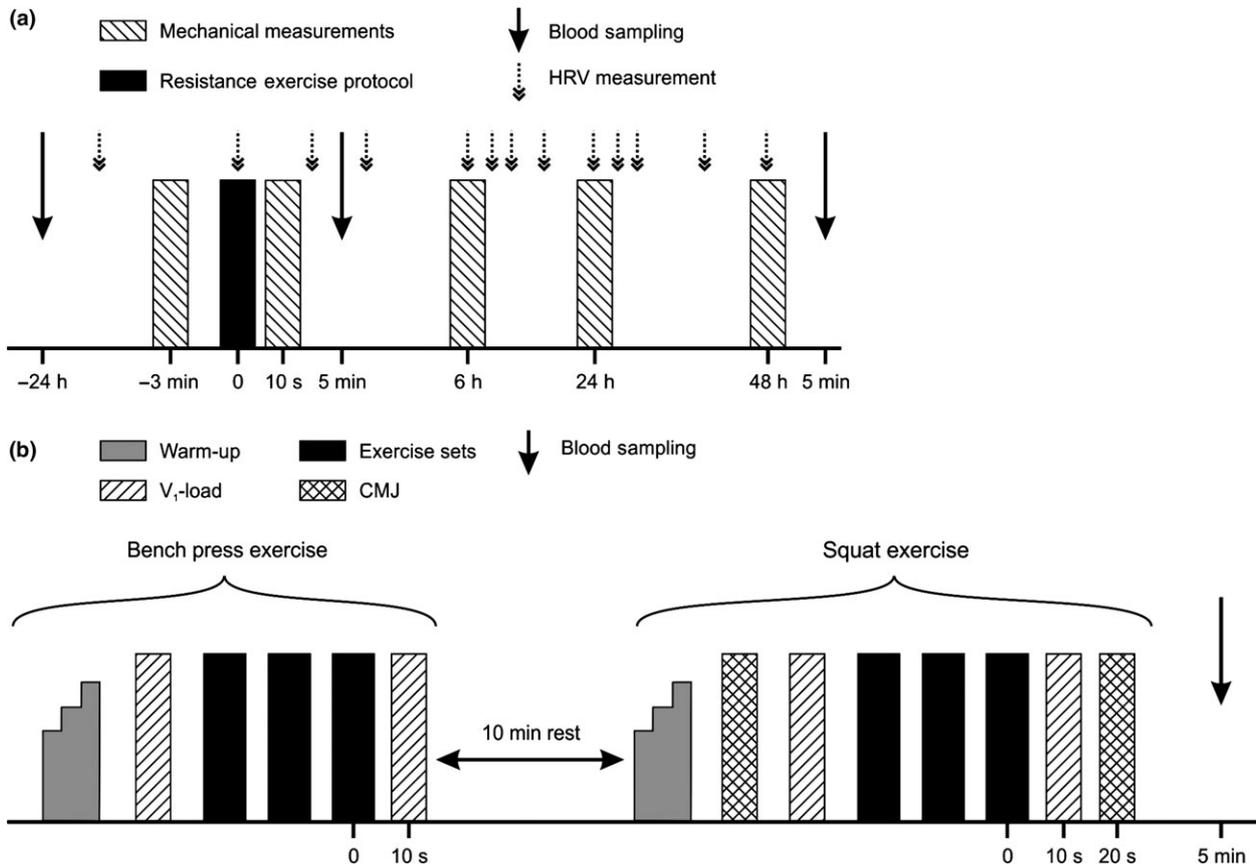


Figure 1 Schematic representation of study design. (a) Mechanical, biochemical and HRV measurements at different time points to analyse the time course of recovery following exercise; (b) Structure of each resistance exercise protocol.

supervised by researchers, and attention was paid to proper exercise lifting technique and instruction on testing procedures. An initial strength assessment was performed 1 week before the first trial.

Procedures

Initial strength assessment

A Smith machine with no counterweight mechanism (Multipower Fitness Line, Peroga, Murcia, Spain) was used for all testing and exercise sessions. Individual load–velocity relationships and 1RM strength were determined using a progressive isoinertial loading test in the BP and SQ exercises, in that order. From the load–velocity relationship, the individual load corresponding to 70% 1RM was determined for each subject and exercise. The velocity corresponding to this load was $0.63 \pm 0.03 \text{ m s}^{-1}$ for BP and $0.82 \pm 0.04 \text{ m s}^{-1}$ for SQ, which agrees with previous research (González-Badillo & Sánchez-Medina, 2010; Sánchez-Medina & González-Badillo, 2011; Pallarés et al., 2014; Pareja-Blanco et al., 2014). The BP was performed imposing a momentary pause (~1.5 s) at the chest between the eccentric and concentric actions to minimize the contribution of the rebound effect and allow for more reproducible, consistent measurements (Pallarés et al., 2014).

The SQ was performed starting from the upright position with the knees and hips fully extended. Each subject descended in a continuous motion until the top of the thighs got below the horizontal plane, then immediately reversed motion and ascended back to the upright position. Subjects were required to always execute the concentric phase of either BP or SQ in an explosive manner, at maximal intended velocity. This execution technique for the BP and SQ was exactly reproduced on the two REP under study. Repetitions were recorded with a linear velocity transducer (T-Force System, Ergotech, Murcia, Spain). Reliability of this system has been reported elsewhere (Sánchez-Medina & González-Badillo, 2011). All reported repetition velocities in this study correspond to the mean concentric velocity of the propulsive phase (Sánchez-Medina et al., 2010).

Acute resistance exercise protocols

Both REP were performed in the morning (10 AM) and were comprised of the BP followed by the SQ, with a 10-min rest between exercises. This order was chosen to avoid the fact that the higher metabolic stress associated with the SQ exercise (greater amount of muscle mass involved) compared with the BP (Sánchez-Medina & González-Badillo, 2011) could negatively influence performance in the latter exercise. Subjects warmed up for the BP by performing 3 min of upper-body

joint mobilization exercises and two sets of eight repetitions with a 20 kg barbell. The warm-up for the SQ consisted of: (i) 5 min of jogging at a self-selected easy pace, (ii) two 30-m running accelerations, (iii) two sets of 10 squats with no external load (own body weight) and (iv) five CMJ with increasing intensity. Then, three maximal CMJ, separated by 20-s rests, were performed and the mean jump height (cm) taken as the preexercise reference value. CMJ height was determined using an infrared timing system (Optojump, Microgate, Bolzano, Italy). In both BP and SQ, the determination of the V_1 -load followed. For this purpose, three sets of six down to three repetitions (2-min inter-set rests) with increasing loads up to each subject's V_1 -load were performed. The mean velocity of the three maximal intended repetitions with the V_1 -load in each exercise was registered as the preexercise reference value for this variable, determined to a precision of $\pm 0.03 \text{ m s}^{-1}$. Finally, load was progressively increased (in 2–3 sets of 3 repetitions each) up to 70% 1RM. The three sets of the corresponding REP were performed next. Immediately after completing the last repetition of the third set (load was changed in 10–15 s with the help of trained spotters), subjects performed three repetitions again with the V_1 -load. Furthermore, after the SQ exercise, another three maximal CMJ, separated by 20-s rests, were performed (Fig. 1b). The V_1 -load and CMJ mean values were taken as the immediate postexercise measures. Strong verbal encouragement and velocity feedback in every repetition was provided throughout all exercise sets.

At 4 PM in the evening (6 h-Post), and at 10AM the following 2 days (24 h-Post and 48 h-Post), the V_1 -load and CMJ measurements were repeated, exactly as described above, to assess the state of neuromuscular recovery following each REP.

Mechanical measurements of fatigue

Three different methods were used to quantify the extent of neuromuscular fatigue induced by each REP (Sánchez-Medina & González-Badillo, 2011). The first method analysed the decline in repetition velocity during the three consecutive exercise sets and was calculated as the percentage loss in mean propulsive velocity from the fastest to the slowest repetition of each set and averaged over the three sets. The second method examined the pre-post exercise change in velocity attained against the V_1 -load. The third method analysed the change in CMJ height pre-post exercise.

Blood collection and analysis

Blood sampling took place 24 h before (Pre), 5 min after completion of the corresponding REP (Post), and 48 h-Post. Subjects rested seated for 30 min before the first blood collection. Samples were drawn from an antecubital forearm vein using a 20-gauge needle connected to vacutainers[®]. The Pre (baseline) samples were drawn at the same time of day (± 15 min) that the REP (10 AM) to minimize the bias in hormonal values due to the circadian rhythm. Whole blood

was centrifuged at 2045 g (4°C) for 15 min and the resultant serum was then removed and stored at -20°C . Samples were assayed in duplicate, thawed only once, and decoded only after the analyses were completed (i.e. blinded analysis procedure). Concentrations of total testosterone, cortisol, GH, PRL and CK were measured using electrochemiluminescence immunoassays on the Elecsys 2010 autoanalyser (Roche Diagnostics, Indianapolis, IN, USA). IGF-1 was measured by chemiluminescent immunometric assay on the Immulite 2000 System (Siemens, Los Angeles, CA, USA). For testosterone, cortisol, GH, PRL, IGF-1 and CK, assay sensitivities were $0.087 \text{ nmol l}^{-1}$, 8.5 nmol l^{-1} , $0.03 \text{ } \mu\text{g l}^{-1}$, $20 \text{ } \mu\text{g l}^{-1}$, $0.047 \text{ } \mu\text{g l}^{-1}$ and 45 IU l^{-1} ; with an intra-assay coefficient of variation of 2.0%, 1.7%, 2.3%, 2.9%, 1.3% and 1.8% respectively. Concentrations are reported uncorrected for plasma volume changes because it has been previously demonstrated that receptors in target tissues are exposed to serum hormonal levels (Rubin et al., 2005).

Analysis of the R-R interval time series

Measurement of consecutive R-R intervals was used as representative of the autonomic nervous system output. R-R intervals were collected using a HR recorder (Firstbeat Bodyguard, Firstbeat Technologies Ltd., Jyväskylä, Finland). This device attaches directly to cleaned skin with two-lead Ag/AgCl electrodes and starts recording data automatically. Subjects wore the recorder uninterruptedly from 24 h before to 48 h post-exercise. Noise and ectopic heart beats were identified and automatically eliminated by the acquisition software. Data were also inspected visually for possible artefacts. Analysis algorithms for all variables analysed were implemented in Matlab 7.11 R2010b (The MathWorks Inc., Natick, MA, USA).

For the time domain analysis, the natural logarithm of the root mean square of successive differences in R-R intervals (LnRMSSD) was calculated (Karavirta et al., 2013; Plews et al., 2013). HR complexity was measured using the complex index (CI) (Costa et al., 2008; Karavirta et al., 2013) computed by summing Sample Entropy (SampEn) values for scales 1–5 (CI_{1-5}). The time series extended to 3 days, from 24 h pre- to 48 h postexercise. Each time series was divided into temporal segments of $3 \cdot 10^3$ data points, and once we coarse-grained them up to scale 5, the shortest time series had 600 points. These temporal segments were as follows: (1) during sleep, the night before the REP (when HR was at its lowest); (2a) during the REP (from the highest measured HR backwards), (2b) immediately postexercise, (2c) in the recovery phase after the REP; (3a) at 6 h-Post, during the V_1 -load and CMJ assessments, (3b) immediately postassessment, (3c) in the recovery phase after the 6 h-Post assessment; (4) during sleep, the night after the REP; (5a) at 24 h-Post, during the V_1 -load and CMJ assessments, (5b) immediately postassessment, (5c) in the recovery phase after the 24 h-Post assessment; (6) during sleep, two nights after the REP; and (7) at 48 h-Post, during the V_1 -load and CMJ assessments.

Statistical analyses

Values are reported as mean \pm standard deviation (SD). Statistical significance was established at $P < 0.05$. Homogeneity of variance across groups was verified using the Levene's test. The distribution of each variable was examined with the Shapiro-Wilk normality test. A factorial ANOVA with repeated measures with Bonferroni adjustment was used to examine the effects of the two REP across time on mechanical, biochemical and autonomic cardiovascular responses. Statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

All variables were normally distributed and homoscedasticity across exercise protocols was verified. No significant differences between 3×12 [12] and 3×6 [12] were found at Pre for any of the variables analysed. As an outlier was observed for baseline CK concentration, this value was omitted in the biochemical analysis.

Descriptive characteristics of the resistance exercise protocols

In the 3×6 [12] protocol, subjects were able to complete all repetitions with the assigned load, whereas during 3×12 [12], most of the subjects could not complete the 12 repetitions due to fatigue (Table 1). Thus, the number of repetitions decreased for 3×12 [12] as sets progressed: 1st set: 11.7 ± 0.7 , 2nd set: 11.5 ± 0.7 , 3rd set: 10.5 ± 2.0 repetitions for SQ; 1st set: 11.6 ± 0.7 , 2nd set: 10.4 ± 1.6 , 3rd set: 9.5 ± 2.1 repetitions for BP.

Mechanical measurements of fatigue

Velocity loss within a set was significantly higher for 3×12 [12] compared with 3×6 [12] for both exercises (Table 1). Velocity against the V_1 -load was significantly reduced for 3×12 [12] versus 3×6 [12] up to 24 h-Post (BP) and 6 h-Post (SQ) (Fig. 2). The 3×12 [12] protocol resulted in significantly lower jump performance than 3×6 [12] up to 48 h-Post (Fig. 3).

Biochemical response

Pre concentrations were within the normal range for physically active young men. Cortisol was higher for 3×12 [12] versus 3×6 [12] at Post ($P < 0.01$, Fig. 4a), but no difference was found between protocols at 48 h-Post. Cortisol levels were significantly lower ($P < 0.05$) at 48 h-Post compared with Pre for both REP. PRL concentration was higher for 3×12 [12] versus 3×6 [12] at Post ($P < 0.01$, Fig. 4b). Higher CK levels were observed for 3×12 [12] compared with 3×6 [12] at 48 h-Post ($P < 0.01$, Fig. 4c), whereas no difference

between protocols was found at Post. Higher IGF-1 levels were observed at Post for 3×6 [12] compared with 3×12 [12] ($P < 0.05$, Fig. 4e). No differences in hormone levels were observed between protocols for GH and testosterone (Fig. 4d,f).

Analysis of the R-R interval time series

As some data were lost due to problems with the HR recording device, only HRV data from seven subjects were analysed. The behaviour of resting HR did not show any significant difference between REP for any of the variables analysed. 3×12 [12] resulted in lower values ($P < 0.01-0.05$) for LnrMSSD, SampEn and CI_{1-5} at immediately Post (2b) than 3×6 [12] (Fig. 5). 3×12 [12] also resulted in lower LnrMSSD at 6 h-Post (3a) ($P < 0.05$). Significant differences ($P < 0.05$) between REP were observed for LnrMSSD at 48 h-Post (7).

Discussion

The main finding of the present study was that the extent of fatigue incurred during RT (going to failure versus ending several repetitions before failure in each exercise set) had a differential effect on the neuromuscular, autonomic cardiovascular and hormonal response to exercise and the subsequent recovery. Compared with 3×6 [12], the 3×12 [12] protocol resulted in a significantly higher velocity loss in both the SQ (44% versus 20%) and BP (65% versus 26%) exercises. In addition, 3×12 [12] resulted in greater muscle damage (CK) and an increased hormonal response (cortisol and PRL). The 3×12 [12] protocol was also associated with a greater decrease in HRV and HRC, and higher neuromuscular fatigue (CMJ height loss and impaired performance against the V_1 -load in BP and SQ). Interestingly, CMJ height did not return to preexercise values at 48 h-Post following 3×12 [12], whereas for 3×6 [12], initial CMJ performance was already recovered at 6 h-Post (Fig. 3). This reduction in the ability to rapidly apply force with the lower limbs up to 48 h following resistance exercise to failure might negatively interfere with the development of other components of physical fitness such as aerobic endurance in sports characterized by concurrent training (Izquierdo-Gabarren et al., 2010; García-Pallarés & Izquierdo, 2011).

It is interesting to note that, even with the ample inter-set rests used (5 min), the number of performed repetitions decreased throughout sets in the 3×12 [12] protocol; i.e. neuromuscular fatigue from previous sets prevented subjects from performing all scheduled repetitions in subsequent sets and forced them to unintentionally reduce repetition velocity. Despite the greater training density and time under tension experienced, which might favour hypertrophic adaptations, training using this type of exhaustive protocols, where the last repetitions of each set are performed at very slow velocities, may considerably increase the amount of time needed for

Table 1 Descriptive characteristics of each resistance exercise protocol.

| | Squat | | | Bench press | | |
|--------------------------------|-------------|-------------|---------|-------------|-------------|---------|
| | 3 × 6 [12] | 3 × 12 [12] | P-value | 3 × 6 [12] | 3 × 12 [12] | P-value |
| Repetitions per set | 6.0 ± 0.0 | 11.2 ± 0.9 | <0.001 | 6.0 ± 0.0 | 10.5 ± 1.3 | <0.001 |
| Fastest-V (m s ⁻¹) | 0.85 ± 0.03 | 0.84 ± 0.03 | 0.47 | 0.65 ± 0.03 | 0.63 ± 0.05 | 0.21 |
| Slowest-V (m s ⁻¹) | 0.63 ± 0.07 | 0.38 ± 0.07 | <0.001 | 0.44 ± 0.05 | 0.15 ± 0.05 | <0.001 |
| Mean-V (m s ⁻¹) | 0.75 ± 0.04 | 0.63 ± 0.07 | <0.001 | 0.55 ± 0.03 | 0.42 ± 0.04 | <0.001 |
| MeanLoss-V (%) | 20.3 ± 6.1 | 43.8 ± 6.7 | <0.001 | 26.4 ± 6.7 | 65.0 ± 5.7 | <0.001 |
| MaxLoss-V (%) | 25.9 ± 8.5 | 54.7 ± 7.4 | <0.001 | 33.4 ± 7.5 | 76.5 ± 7.3 | <0.001 |

Fastest-V, highest velocity measured in the three sets; Slowest-V, lowest velocity measured in the three sets; Mean-V, mean velocity of all repetitions during the three sets; MeanLoss-V, mean percentage loss in velocity from the fastest to the slowest repetition over the three sets; MaxLoss-V, maximum percentage loss in velocity from the fastest to the slowest repetition over the three sets.

Data are mean ± SD, n = 10.

Velocities correspond to the mean concentric propulsive velocity of each repetition.

P-value indicates the magnitude of the significance between the two resistance exercise protocols, 3 × 6 [12] versus 3 × 12 [12].

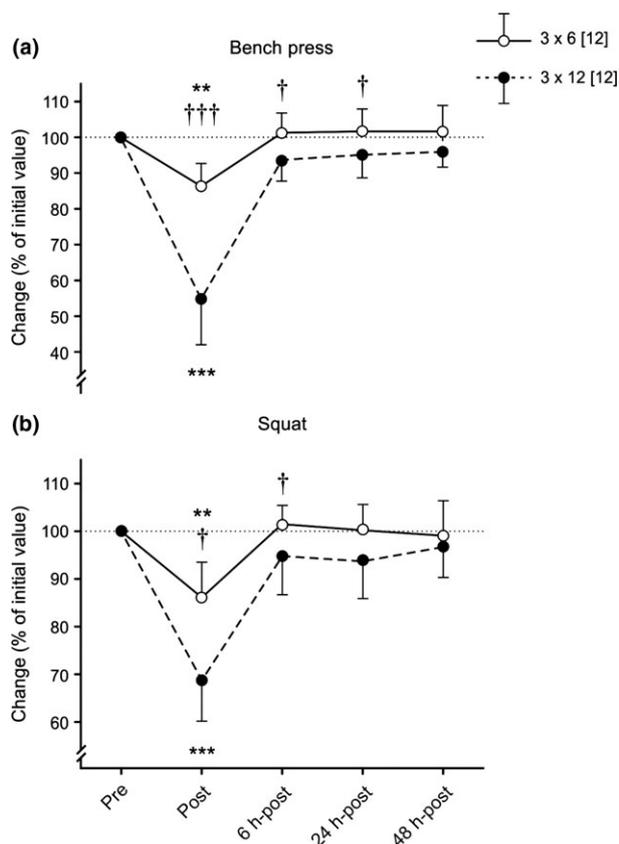


Figure 2 Comparison of the changes in the velocity attained against the V_1 -load between the two REP at the different time points for (a) Bench Press; and (b) Squat exercises. Changes are expressed as percentage of the initial, preexercise, baseline values. Statistically significant differences between REP (3 × 6 [12] versus 3 × 12 [12]) at the corresponding time point: † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$. Statistically significant differences with Pre at the corresponding time point: ** $P < 0.01$, *** $P < 0.001$.

recovery, as previously suggested (Sánchez-Medina & González-Badillo, 2011). Inadequate or insufficient recovery after training could result in fatigue or underperformance

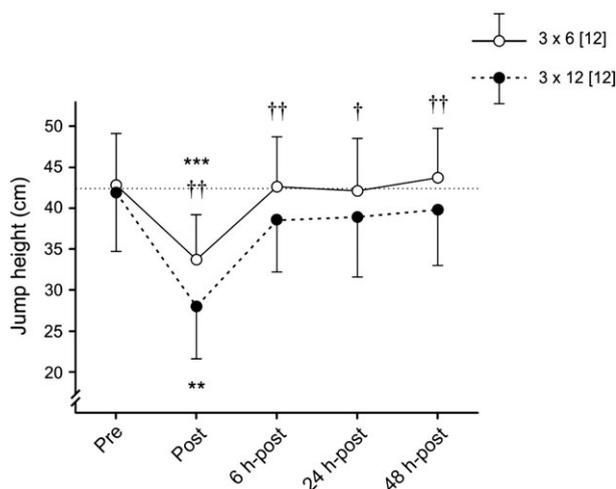


Figure 3 Comparison of the absolute changes in CMJ height between the two REP at the different time points. Statistically significant differences between REP (3 × 6 [12] versus 3 × 12 [12]) at the corresponding time point: † $P < 0.05$, †† $P < 0.01$. Statistically significant differences with Pre at the corresponding time point: ** $P < 0.01$, *** $P < 0.001$.

(Chen et al., 2011). It has been reported that performing repetitions to failure causes a marked disruption in the muscle energy balance and a considerable depletion of muscle purines, whereas performing a half-maximal number of repetitions per set allows the maintenance of cellular homeostasis (Gorostiaga et al., 2012, 2014). These findings may explain the incomplete recovery observed in the hours following the 3 × 12 [12] protocol, as the replenishment of the muscle adenine nucleotide pool is a time-consuming process that may take up to several days to complete (Stathis et al., 1999). It thus seems reasonable to think that in the hours following a REP such as 3 × 12 [12], a person's physiological environment would not be in optimal conditions to assimilate more training or improve neuromuscular performance. These results are even more compelling if we consider that only two

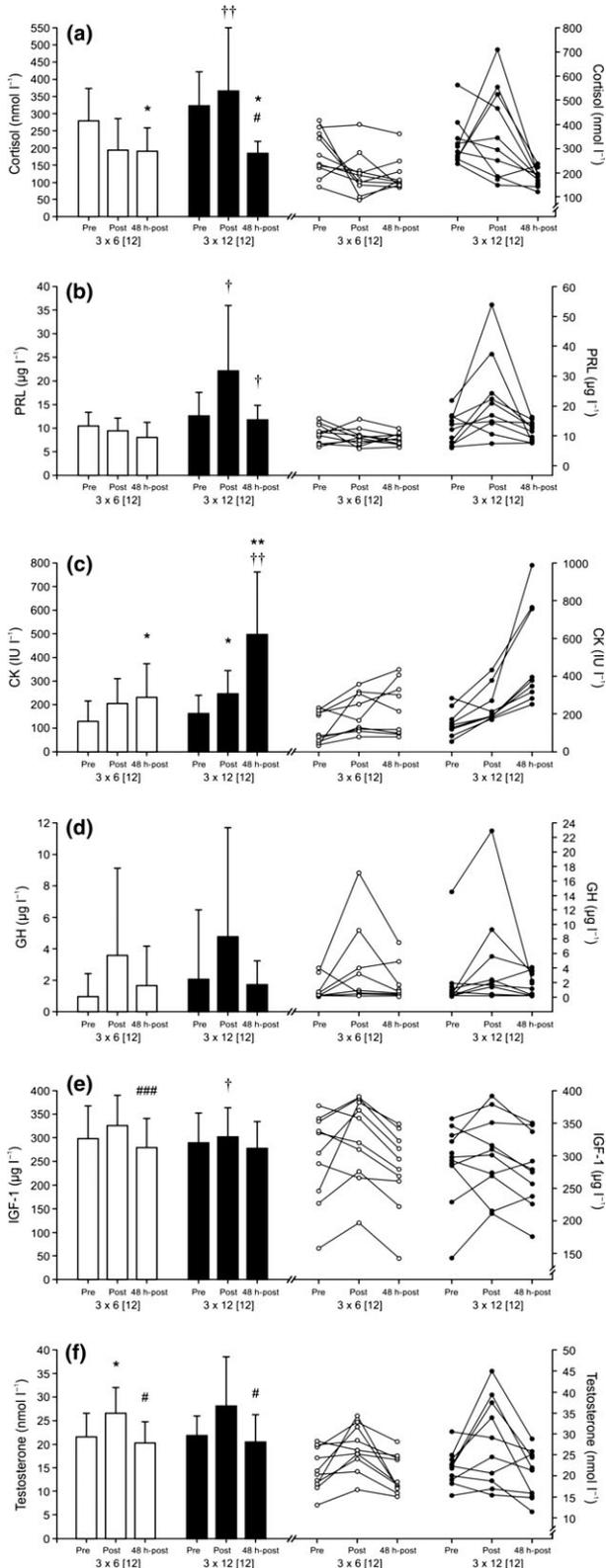


Figure 4 Changes in the concentration of the biochemical markers analysed at the three measurement time points (Pre, Post and 48 h-Post): (a) Cortisol; (b) Prolactin; (c) Creatine Kinase; (d) Growth Hormone; (e) Insulin-like Growth Factor-1; (f) Testosterone. Mean and SD (left) as well as individual values (right) are shown for each graph. Statistically significant differences between REP (3 × 6 [12] versus 3 × 12 [12]) at the corresponding point: †*p*<0.05, ††*p*<0.01. Statistically significant differences with Pre at the corresponding time point: **p*<0.05, ***p*<0.01. Statistically significant differences with Post at the corresponding time point: #*p*<0.05, ##*p*<0.01, ###*p*<0.001.

recovery following different organization schemes for a RT training session (number and order of the exercises) is warranted.

It has been reported that mechanical tension, metabolic and hormonal stress as well as muscle damage mediate hypertrophic adaptations (Schoenfeld, 2010). Thus, the

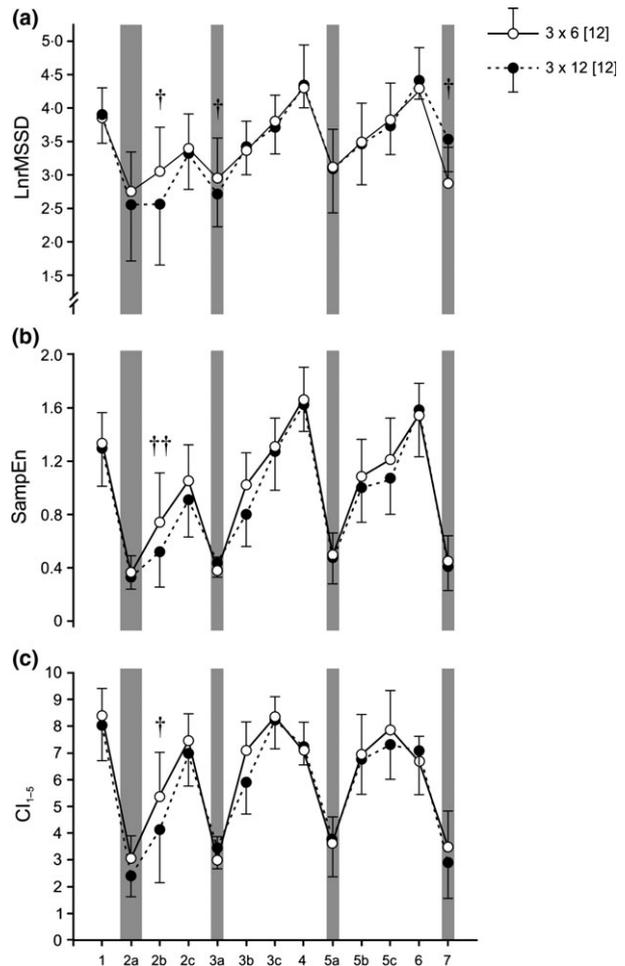


Figure 5 Time course of the three HRV variables analysed following each REP. (a) Natural logarithm of the root mean square of differences in R-R intervals (LnRMSSD); (b) Sample Entropy (SampEn); (c) Complexity Index (CI₁₋₅). Thirteen temporal segments (1, 2a, 2b, 2c, 3a, 3b, 3c, 4, 5a, 5b, 5c, 6, 7) covering from 24 h preexercise to 48 h-Post were established (see Methods for details). Vertical shaded areas indicate the segments corresponding to exercise. Statistically significant differences between REP (3 × 6 [12] versus 3 × 12 [12]) at the corresponding temporal segment: †*p*<0.05, ††*p*<0.01.

exercises (BP and SQ) were performed in each REP, whereas typical RT sessions are usually more demanding and consist of at least 5–8 exercises. Research analysing the time course of

higher muscle damage and hormonal response observed for the 3×12 [12] REP might lead to greater hypertrophy. The biochemical mechanisms responsible for the observed increases in blood hormonal concentrations are yet not fully understood and are beyond the scope of the current study. However, the high levels of blood lactate and H^+ with the consequent pH decrease, together with the significant depletion in phosphocreatine stores and total adenine nucleotide pool reported during RT to failure (Sánchez-Medina & González-Badillo, 2011; Gorostiaga et al., 2012), might explain the greater stress-related hormonal response (PRL and cortisol) observed for 3×12 [12]. In this regard, a higher cortisol response has been observed following RT protocols that included forced repetitions to increase total training volume (Ahtiainen et al., 2003). The greater cortisol and CK concentrations following 3×12 [12] are in agreement with previous studies that reported a significant correlation between cortisol and CK levels (Boone et al., 1990; Kraemer et al., 1993). Although absolute values of IGF-1 remained basically unaltered for both REP in the present study, 3×6 [12] resulted in greater IGF-1 concentration at Post than 3×12 [12], a finding that is difficult to explain. On the other hand, testosterone showed a trend to increase (24–29%) at Post following both protocols, similar to that reported in other studies that performed repetitions to failure (Hakkinen & Pakarinen, 1993; Ratamess et al., 2005). Despite some research evidence that the magnitude of the testosterone response depends on the stress of the RT session (Hakkinen & Pakarinen, 1993), our results suggest that both REP, 3×6 [12] and 3×12 [12], might have reached the threshold needed to elicit elevations in testosterone of similar magnitude. In addition, both REP increased GH at Post, with a tendency for 3×12 [12] to result in greater levels of this hormone. It is known that exercise that imposes great demands on glycolysis, thus resulting in marked increases in H^+ concentration, may be the primary physiological stimulus for GH release (Gordon et al., 1994).

There is a growing body of literature aiming to analyse the acute effects of RT on HRC (Heffernan et al., 2007, 2008; Iglesias-Soler et al., 2015; Kingsley & Figueroa, 2014). These studies have used only the SampEn variable to analyse the HRC, an algorithm that quantifies the degree of irregularity on the shortest timescale, but fails to quantify it on longer timescales (Costa et al., 2006). In the present study, in addition to SampEn, we analysed the CI_{1-5} using the multiscale entropy method to quantify the magnitude of irregularity over a broader range of timescales. Both 3×12 [12] and 3×6 [12] protocols resulted in acute decreases in HRV and HRC following exercise (Fig. 5). However, 3×12 [12] showed higher reductions in HRV and HRC than 3×6 [12], which seems to correspond with the greater level of neuromuscular and hormonal stress induced by 3×12 [12]. De Souza et al. (2013) compared the effects of three sets of 10RM, and three sets of 10 repetitions at 60% of 10RM on 24 h cardiovascular response in adult women. Root mean square of standard deviation (rMSSD) decreased ($P \leq 0.05$) after both protocols, without significant differences between protocols. However, De Souza et al. (2013) compared exercise protocols of equal volume while manipulating loading, whereas in the present study, loading magnitude (%1RM) was the same for both protocols, but exercise volume changed. Loss of HRC has been associated with inflammation (Rassias et al., 2005). Inflammation is a symptom of the mechanical stress affecting muscle structures. As 3×12 [12] induced an impairment in mechanical performance (velocity against the V_1 -load and CMJ height losses) and was associated with greater physiological stress (higher CK, cortisol and PRL postexercise levels), this could have influenced the more pronounced decrease in HRV and HRC observed following this REP in the present study.

As a limitation of this study, we must acknowledge that the link between the concomitant *in vivo* changes of endogenous anabolic hormones and tissue growth has not been specifically determined, and thus increased concentrations of circulating anabolic hormones may not reflect anabolism at the tissue level. Other factors such as hormonal clearance rates, hormone degradation, receptor binding protein activation and regulation should also be examined in detail to gain a better insight into the effects of the degree of fatigue incurred during each resistance exercise set.

In conclusion, the present study provides novel findings about the mechanical, biochemical and autonomic cardiovascular response to two REP only differing in the number of performed repetitions per set with regard to the maximum possible (to failure) number that can be completed. Our results seem to indicate that RT to failure induces greater mechanical fatigue (assessed by the impairment in neuromuscular performance throughout the postexercise recovery period), greater autonomic cardiovascular stress (higher reductions in HRV and HRC) and an increased hormonal response and muscle damage. The performance impairment observed in CMJ height up to 48 h postexercise following the 3×12 [12] protocol may indicate that the 'explosiveness' or ability to rapidly develop force with the lower limbs may be considerably compromised up to 48 h following resistance exercise to failure. As a practical application for the strength and conditioning coach, the CMJ test could be implemented as an easy, cheap and quick method to assess the state of neuromuscular recovery, something which has already been suggested (Gorostiaga et al., 2010). Furthermore, both REP induced very different repetition velocity losses in the SQ (44% versus 20%) and BP (65% versus 26%) exercises for 3×12 [12] versus 3×6 [12] respectively. Monitoring repetition velocity and setting a certain percentage velocity loss limit (e.g. 20%, 25% or 30%) to stop an exercise set is another practical measure that can be implemented to avoid performing unnecessarily slow and fatiguing repetitions that may not be contributing to the desired training effect. This rational alternative to traditional RT can be used to better monitor and individualize the training load, and it can be accomplished by means of the ever-

increasing number of commercially available linear position or velocity transducers and accelerometers. Taken together, the findings of the present study might provide useful information for implementing a comprehensive RT programme in many sports where the performance goal is not merely focused on maximizing muscle hypertrophy or improving maximal strength, but rather it is necessary to develop specific neuromuscular adaptations while trying to avoid excessive fatigue that could interfere with the development of other components of physical fitness or negatively affect the technical, tactical or recovery aspects of training.

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Conflict of interest

The authors declare no conflicts of interest.

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