DIFFERENTIAL IL 10 SERUM PRODUCTION BETWEEN AN ARM-BASED AND A LEG-BASED MAXIMAL RESISTANCE TEST

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Highlights

- Elevated serum levels of IL10 were detected in the arm-based exercise.
- Both exercises induced similar IL6 and TNFα serum levels.
- The arm-based resistance test induced a higher muscular damage than the leg-based test.

Abstract

Bench press (i.e. arm-based) and half-squat (i.e. leg-based) are exercises commonly used to increase and evaluate muscular strength. In addition to differences in the location of the muscles that participate in each exercise, the total muscle mass required for the latter is larger than that involved in the former. The aim of this study is to analyze the effects of a maximal incremental strength test when performed by bench press and by half-squat on myocellular damage, oxidative damage and the inflammatory cytokine response. Ten male athletes were subjected to half-squat and bench press incremental strength tests. Blood samples were collected at rest, 15-minutes and 24 hours post-test. Hydroperoxide and malondialdehyde concentrations were determined as lipid peroxidation markers. Lactate dehydrogenase (LDH) and creatine kinase isoenzyme MB (CK-MB) activities were determined as markers of muscle damage. α-Actin concentration was determined as a marker of sarcomeric damage. Serum interleukin (IL) 6, IL10, and tumor necrosis factor alpha (TNFα) were determined to assess the inflammatory response. LDH and CK-MB values were greater at 15 minutes and 24 hours post bench press exercise ($p < 0.05$). No differences were found in lipid peroxidation or α-actin. Interestingly, IL10 values were greater in response to the press bench at 24 hours post-test ($p < 0.05$). Our results suggest that, at equivalent workloads,

an arm-based exercise induced higher anti-inflammatory effects and more severe muscle damage compared with a leg-based exercise.

Keywords: IL10; serum interleukins; inflammatory response; lipid peroxidation; resistance training.

Abbreviations: CK-MB, creatine kinase isoenzyme MB; LDH, lactate dehydrogenase; IL, interleukin; TNFα , tumor necrosis factor alpha; ROS, reactive oxygen species; 1RM, one-repetition maximum; HPX, Hydroperoxides; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substances; ANOVA, a two-way mixed analysis of variance; η^2p, partial eta squared; IKAK, International Society for the Advancement of Kinanthropometry; SEM, standard error of the mean; MAPK, mitogen-activated protein kinase (MAPK).

1. Introduction

Regular resistance training is considered an important intervention in order to maintain a healthy lifestyle. In fact, resistance exercise can prevent several age-related metabolic diseases such as sarcopenia and insulin resistance [1], and is well known to produce a particular response in the immune system [2]. A single series of resistance-exercise affects in a transient manner leukocyte trafficking and cell functionality [3]. Moreover, monocytes and lymphocytes are released after resistance-exercise in an effort-dependent fashion [4]. In fact, different immune responses may be triggered depending on several factors such as the nature of the performed exercise, the intensity, the recruited muscle-mass volume (sets x repetitions) or the duration and rest periods

(recovery between sets) [2]. Therefore, controlling resistance training is of importance both for health and performance-related purposes.

It is known that exercise activates an immune response, although of varying effects [2]. According to Nieman et al., there is a dual relationship between the exercise workload continuum and risk for upper respiratory tract infection [5]. In fact, moderate intensity exercise reduces the probability of suffering from any kind of infection (40 or 50%). However, vigorous exercise increases the probability of infection up to 6 times [5]. After moderate-intensity exercise, immune cells such as monocytes, lymphocytes, natural killers and neutrophils, are redistributed more efficiently between blood and lymphoid tissues, increasing the immunosurveillance. The level of anti-inflammatory cytokines and immunoglobulins increases in parallel with a decrease of pro-inflammatory cytokines and others hormones [5]. In fact, a comparative study reported that the lower dose of resistance-exercise did not alter the monocyte count [6]. Conversely, the increment of intensity and/or duration of exercise tended to alter the immune response [2]. Markers associated with this immune dysfunction are found after prolonged and intensive exercise, indicative of inflammation, oxidative stress, and muscle damage. Multiple disturbances in T and B lymphocyte function, MHC-II complex in macrophages and skin hypersensitivity response could explain the high risk of infections and other diseases in the respiratory tract associated with the intense and prolonged exercise [5].

Muscle contraction induces a transient production of reactive oxygen species (ROS) [9]. Within the untrained skeletal muscle, ROS production can exceed the capacity of both enzymatic and non-enzymatic antioxidant defense leading to myocellular damage [10]. These events lead to a rapid release of inflammatory cytokines such as TNFα [11]. Concomitantly, skeletal muscle secretes IL6 which helps

to counteract such a pro-inflammatory response by targeting IL10 [12,13] which, in turns, blocks TNFα expression [14]. However, the immune response between the upper and lower-body musculature during an acute resistance exercise remains unclear.

An important issue when controlling resistance exercise is the fact that lower-body musculature (i.e. legs) has a more oxidative phenotype than the upper-body musculature (i.e. arms) [7], which may likely explain the fact that upper-body musculature shows a shorter recovery than lower-body musculature from a single resistance session [8]. Moreover, the latter study showed that arm exercise tends to induce a greater degree of myocellular damage than legs [8]. However, whether the metabolic response differs between an arm-based and a leg-based resistance exercise is unknown.

Two commonly used arm-based and leg-based resistance exercises are bench press and half-squat, respectively [15]. In both exercises, the force is produced through the combination of eccentric, concentric, and joint-stabilizing isometric muscle actions which form basic components of the movements performed in daily living [16]. In addition, they are frequently used to assess muscular strength [17]. Thus, bench press and half-squat can be used both by athletes and by sedentary subjects in order to control resistance training. Therefore, the present study aimed to describe the effects of two maximal incremental strength tests (performed by bench press and by half-squat) on myocellular damage, oxidative damage and production of cytokines involved in the inflammatory response.

2. Methods

2.1. Subjects

A total of 10 well-trained male participants familiarized with half-squat exercise and bench press enrolled voluntarily in the study. All participants had recreational experience (1 to 5 years) in both exercises and trained their body musculature 2-3 times per week by lifting loads of 60-90% of one-repetition maximum (1RM) [18]. Through the use of a questionnaire and a personal interview, smoking, high intake of antioxidant-rich foods, ergogenic supplementation or drug consumption were considered exclusion criteria. Subjects were thoroughly informed of the purpose, nature, practical details and possible risks associated with the study before they provided their written informed consent to participate. The study was conducted in accordance with the Declaration of Helsinki (2000) and it was approved by the University of Granada's Ethics Committee on Human Research, (Number 417).

2.2. Design

Subjects were assessed in the morning, two hours post-prandial, at a laboratory with controlled humidity and temperature according to the guidelines established by the National Strength and Conditioning Association (NSCA-National Strength & Conditioning Association 2008).

All subjects refrained from vigorous physical activity during the 96 hours prior to each test. Pre-test sessions were conducted 2 weeks before the experimental trials, under the supervision of an expert investigator. Three days before the experiment, the subjects received dietary recommendations by a nutritionist. In addition, the diet eaten was recorded and subsequently evaluated. All tests were performed on a Smith Machine

(Life Fitness, Brunswick Corporation, Chicago, IL), which has a barbell attached to stationary vertical supports to restrict the barbell motion within the frontal plane [19]. The first week, subjects underwent an anthropometric assessment and were familiarized with the experimental testing procedures. The second week was dedicated to the assessment of the participants' 1RM in both exercises. The tests were performed on different days allowing a 5-day resting period in between.

Finally, participants performed maximal incremental strength tests for both bench press and half-squat exercises, allowing a 14-day resting period between tests. The exercises were performed in a random order. Half of the subjects performed the half-squat exercise first, whereas the other half performed the bench press exercise first. The following week, the subjects switched the order of the exercises. Four days of experiment were required to evaluate the subjects. During each test, blood samples were collected at rest, 15-minutes, and 24 hours post-test.

2.3. Anthropometry

All participants underwent an anthropometric assessment following the protocol developed by the International Society for the Advancement of Kinanthropometry (ISAK) [20].

2.4. One repetition maximum (1RM) strength testing sessions

All the participants underwent two tests to determine their 1RM for half-squat and bench press exercises, respectively, following the methodology described by Fernández-Gonzalo et al [21]. Before the test, subjects performed a warm-up, which included 5 minutes on a cycle ergometer, dynamic stretching and 2 sets of 5 repetitions with 20 and 30 kg, respectively. The initial load was set at 40 kg for bench press, and 90

kg for half-squat. The load was increased by 10 kg when the subjects succeeded, whereas the load was reduced by 5 kg when the subjects failed the task. The test was terminated when the subjects failed to perform two consecutive attempts. All the repetitions were interspersed by a 3-minutes rest and the 1RM was achieved between the third and sixth attempt.

2.5. Maximum incremental strength test

Each subject executed 2 tests, a half-squat and a bench press test, 14 days apart from each other. The subjects were asked to refrain from exercise for a minimum of 96 hours prior to the tests. The standard warm-up described earlier was applied prior to testing. The test used was validated according to Aragón-Vela et al. [22].

During the half-squat test, shoulders were in contact with the bar, and the starting knee angle was 180º. Foot spacing was set at approximately the same width as, or slightly wider than, shoulder width with toes pointing slightly outward [23]. To control the displacement path, the position that the individual needed to initiate the half-squat was determined using an electrogoniometer (TSD130B, Biopac Systems, Inc., CA). The subject was directed to half-squat until touching the rod with the glutei. Eccentric and concentric phases were continuously performed, and knee flexion angle was kept at 90º. The torso was kept as straight as possible and a security belt was used by all subjects [24].

During the bench press test, the head, the shoulders and the hips were supported by the bench with 90º flexion of the knees, as suggested by Cotterman et al. [23]. The barbell was lowered in a continuous motion until the bar position was 1-2 cm above their intermammary line, and they were required to maintain this position for 1s (velocity = 0 m/s). From that position, every participant was instructed to perform a

purely concentric action (as quickly as possible) to return to the initial position [25]. This technique allows an optimal use of the *pectoralis major* while still allowing the triceps to add to initial explosiveness [26]. Press hand spacing was set at 165–200% of bisacromial width, which has been shown to provide the optimal strength values of all grip widths for the supine bench press [27]. Hand and foot spacing were recorded for replication in subsequent tests [23].

In both tests, five intensities derived from the individual 1RM were incrementally used: 20, 40, 60, 80 and 100% 1RM. For the first three intensities, the participants performed 5 repetitions separated by 5-second intervals. At 80% 1RM intensity, the participants performed 2 repetitions 5 seconds apart from each other. Finally, only 1RM was completed by each participant. A 3-minute resting period was allowed after each of the first four intensities (20%, 40%, 60% and 80%), and a 5-minute resting period was allowed between 80% and 100% intensities. Feedback was provided by personnel that timed and guided the athletes during the eccentric and concentric phases, making sure that the concentric phase was always performed explosively at the maximum possible speed [25], and also assisting in both raising the bar on a failed attempt and placing the bar back on the rack.

2.6. Blood sampling

Blood samples were collected at rest as well as 15 minutes and 24 hours post-test. Samples were centrifuged at 3,000 rpm and 4 °C for 15 min to separate serum from cells.

2.7. Lipid Peroxidation

Hydroperoxides (HPX) were measured in serum with the PeroxiDetect kit from Sigma (St. Louis, MO) following the manufacturer's instructions. Briefly, the hydrogen peroxide standard curve and plasma samples (100µl) were mixed with the working color reagent and incubated at room temperature during 30 minutes. After this, absorbance changes at 560 nm were monitored spectrophotometrically.

Plasma thiobarbituric acid (TBAR) concentration is an indirect method of estimating malondialdehyde (MDA) and was measured as described by Orrenius et al. [28]. Plasma samples were lysed and 100 µl of each one were mixed with 200 µl ice cold 10% Trichloroacetic acid to precipitate proteins. After a 15-minute incubation, 200 µl of TBA were added into the supernatants and standards, and incubated 10 min in a boiling water bath. Then, the samples were analyzed spectrophotometrically at 532 nm.

2.8. Serum CK-MB and LDH activities

CK-MB analysis was performed using a commercial kit purchased from Spinreact, S.A following the manufacturer's instructions (Gerona, Spain, ref: TK41255). Briefly, 1 ml of working reagent and 40 µl of sample supernatant were mixed and incubated for 10 min at 37ºC. Absorbance changes at 340 nm were monitored spectrophotometrically.

LDH analysis was performed using a commercial kit purchased from Spinreact, S.A. (Gerona, Spain, ref: 41223). Three 3 ml of working reagent and 50 µl of sample were mixed and incubated 1 min at 37ºC. Absorbance changes at 340 nm were monitored spectrophotometrically, each minute for 3 minutes in total.

2.9. α-Actin quantitation

α-Actin concentration was determined as a marker of sarcomeric damage by western blot as we have recently described by Casuso et al. [29]. A sample of serum (5 µl) from each subject was placed in sample buffer (62.76 mM Tris-HCl pH 6.8, 1% 2-mercaptoethanol, 1% SDS, 10% glycerol, and 0.01% bromophenol blue) at 1:3 ratio, boiled for 10 minutes, microfuged for 10 seconds and analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis in a Mini Protean transfer system (Bio-Rad, Hercules, California, USA) at 60 mA and room temperature for 90 minutes. Proteins were separated electrophoretically and transferred onto a 0.2 µm nitrocellulose membrane (Trans-Blot transfer pack, Bio-Rad) by applying a current of 25V at room temperature for 7 minutes using a Trans-Blot Turbo Transfer system (Bio-Rad). The blots were treated with blocking solution (5% non-fat milk in TBST buffer: 20mM Tris, 0.9 % NaCl) for 1 hour at room temperature. Then, the blots were incubated with the primary antibody (Actin (C-11): sc-1615, 1:1000 dilution, Santa Cruz Biotechnology, Dallas), overnight at 4^{o} C. Membranes were subjected to three 15-minute washes in 5% TBST, incubated with the secondary antibody (bovine anti-goat IgG-HRP: sc-2350, 1:5000 dilution, Santa Cruz Biotechnology, Dallas) for 1 hour at room temperature, and again washed 3 times in TBS-T. Proteins were visualized by enhanced chemiluminescense (SuperSignal West Dura Chemiluminescent Substrate, 34075, Thermo Scientific).

Densitometric analysis was performed by scanning radiographic images of membranes. Image resolution was 100 points per inch. Image treatment software (Adobe Photoshop 5.0, Adobe Systems) was used to treat images, which were saved in TIF format to allow them to be accessed by the software for quantification (Quantity

One 1-D, Bio-Rad). α-Actin was quantified by comparison with a standard curve done with increasing concentrations of pure actin (Sigma) and run in parallel (Figure 1, panel C) [29].

2.10. Cytokine assay

Serum IL6 (# 88-7066-22), IL10 (# 88-7106-22), and TNFα (# 88-7346-22) were measured using commercially available ELISA kits according to the manufacturer's instructions (Thermo Fisher Scientific, Canada). All samples and provided standards were analyzed in triplicate.

Briefly, the Corning™ Costar™ 9018 ELISA plates were coated with 100 µL/well of capture antibody in Coating Buffer. Then, plates were sealed and incubated overnight at 4°C. After aspiration of the supernatant, wells were blocked with 200 µL of ELISA/ELISPOT Diluent (1X) and incubated at room temperature for 1 hours. The wells were again washed and 100 µL/well of samples or standard as well as 100 µL of ELISA/ELISPOT Diluent (1X) were added to the appropriate wells and incubated overnight at 4°C. After aspiration of the supernatants and a new wash, 100 µL/well diluted detection antibody was added to all wells and incubated at room temperature for 1 hr. Plates were incubated with the enzyme and the substrate for 15 minutes at room temperature. Finally, O.D. readings were measured at 450 nm in a plate microreader (Synergy NEO 2, BIOTEK, Izasa Scientific).

2.11. Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). The normality of distribution was assessed by the Shapiro-Wilk test. The homogeneity of variance was analyzed through the Levene test. A two-way mixed analysis of variance (ANOVA)

was used with exercise as the between-subjects factor (half-squat vs bench press) and time as the within-subjects factor (at rest, 15-min post-test, and 24-hours post-test). Multiple comparisons were performed by the Bonferroni post-hoc test. Effect sizes were calculated using partial eta squared (η^2p). The level of significance was set at $p<0.05$. All statistical procedures were carried out using SPSS/PC V. 22 (SPSS Inc., Chicago, IL, USA).

3. Results

A total of 10 well-trained athletes were recruited for this study. Characteristics of these athletes are described in Table 1.

Our data showed a statically significant interaction between exercise and time in CK-MB ($F(1,18) = 6.544$, $p = 0.020$, $\eta^2p=0.271$) and LDH ($F(1,18) = 8.868$, $p= 0.008$, $\eta^2p=0.330$), and therefore, the response of CK-MB and LDH to the maximum incremental strength test was different depending on the type of exercise (Figure 1).

The effect of exercise type presented significant differences in CK-MB ($F(1,18) = 9.981$, $p = 0.005$, $\eta^2p=0.357$) and LDH ($F(1,18) = 7.914$, $p = 0.012$, $\eta^2p=0.305$), with higher activities in arm-based compared with half-squat for both CK-MB ($MD = 6.16 \pm 1.95$, $p = 0.005$) and LDH ($MD = 58.98 \pm 20.96$, $p = 0.012$) (Figure 1). Pairwise comparisons of lipid peroxidation values did not show differences due to exercise (Table 2).

In addition, we found a statistically significant main effect of time in LDH ($F(1,18) = 5.108$, $p = 0.036$, $\eta^2p=0.221$) in half-squat. LDH values showed a significant decrease 24 hours post-test compared with 15-minutes post-test ($MD = 51.82 \pm 18.28$ $p = 0.033$) in half-squat. The rest of the variables did not show significant differences due to time. A significant decrease occurred in CK-MB and LDH at 24 hours post-test

compared with baseline (MD = 86.75 ± 23.42, p = 0.005; MD = 9.33 ± 3.23, p = 0.030, respectively) and 15-min post-test (MD= 74.23 ± 25.86, p = 0.031; MD = 6.22 ± 2.23, p = 0.036, respectively) in half-squat.

Comparisons for TBARS and HPX appear in Table 2. Significant differences neither within time nor between both exercises were found in these variables. On the other hand, the values of IL6 and TNFα did not show significant differences within time or between both exercises. However, we found a statistically significant main effect of time in IL10 ($F(1,18) = 9.278$, $p = 0.007$, $\eta 2=0.340$). IL10 values showed a significant increase at 24 hours post-test compared with 15-minutes post-test (MD = 0.764 ± 0.249 = 0.020) in bench press (Figure 2).

4. Discussion

In the present study we used a randomized crossover study design in order to compare the acute metabolic responses on an arm-based (i.e. bench press) to a leg-based (i.e. half-squat) maximal incremental strength test. Our results show that, with an equivalent workload, bench press exercises induced a greater muscle damage and higher anti-inflammatory response than half-squat. The results are consistent with previous studies, which showed that arm exercises tend to generate greater systemic alterations and greater inflammatory response than leg exercises [8,30]. It is known that after a series of heavy resistance exercise a transient inflammatory response is generated, including an increase in white blood cell count and stimulation of pro- (IL6, TNFα) and anti-inflammatory (IL10) cytokines [31]. However, to our knowledge whether arm-based and leg-based maximal strength tests generate different immune responses has not been described.

Whereas changes in cytokine serum levels following prolonged endurance exercise have been described, serum cytokine variations have not. A marked increase in IL6 and IL10, and a moderate rise in TNFα in systemic circulation occurs in response to prolonged aerobic exercise [11], thus suggesting an overall acute anti-inflammatory effect. In contrast, following a 2 hours of intensive and full-body resistance training the changes in plasma cytokine levels were smaller than the response described for endurance-like exercises [32]. Accordingly, we found that neither bench press nor half-squat altered circulating IL6 or TNFα. The lack of an increment in IL6 could have several explanations. First, it has been reported that 50 (i.e. 5 x 10) repetitions at 70% of 1RM increases plasma IL6 [33]. However, our protocol only comprised 18 repetitions, and therefore it is likely that a more vigorous demand may be needed to modulate plasma IL6 levels. Second, Afroundeh et al. reported elevated IL6 levels during the 24 hours period after resistance training, peaking 8 hours post exercise [34]. Therefore, it cannot be excluded that we may have missed the IL6 peak as the 8 h timepoint was not included in our study. Finally, it is important to note that we studied well-trained subjects which may have higher basal IL6 levels than the average population [4], and this could mask some of the acute responses induced by our resistance protocol. Taken together, the high athletic training of the participants may have contributed to the fact that IL6 was unchanged and this population may need greater training volumes to enhance serum IL6.

The most conspicuous finding of our study is the increment in plasma IL10 in response to bench press exercise. These results are in accordance with those reported by Hirose et al. showing that an eccentric exercise of the elbow flexors induces a systemic anti-inflammatory response by rising circulating IL10 levels [30]. However, the fact that bench press, but not half-squat, rises IL10 circulating levels suggests that arms may

have a highly efficient anti-inflammatory machinery in response to physical stress. Notably, we have recently showed that IL10 increases to a similar extent after a sprint interval training both when performed by swimming and by running [35], thus, suggesting that the underlying mechanism for the differences observed in IL10 between arms and legs is of local rather than of systemic origin.

One explanation for the high anti-inflammatory response of the arms relies on the fact that, at an equivalent workload, arm muscles show a higher degree of damage than legs [8]. Our results showing that both CK-MB and LDH release increases after bench press but not after half-squat are in agreement with this fact. Indeed, the lower CK-MB and LDH values found in leg exercise might be due to a more efficient clearance of enzymes from serum since leg muscles are larger than arm muscles [36]. Thus, it can be argued that the anti-inflammatory response observed after the bench press exercise might be a compensatory mechanism in order to prevent an excessive local inflammation.

Similarly, oxidative stress induced by resistance exercise is another mechanism that may explain the anti-inflammatory response. The resistance exercise has been associated with oxidative damage which can indeed modify the structure of different molecules, such as proteins. When this occurs, such oxidized proteins cease to be functionally active and, therefore, they are unable to exert their normal cellular functions [37]. To avoid this situation during and after resistance exercise, NF-κB and mitogen-activated protein kinase (MAPK) pathways are activated by cellular stress and muscular damage derived stimuli to trigger the transcription of pro-inflammatory cytokines and redox-status regulator molecules [38]. Consequently, the release of anti-inflammatory cytokines, such as IL10 is necessary, in order to alleviate the damaging effect of inflammation and, therefore, to maintain the cellular hemostasis between pro

and anti-inflammatory cytokines. However, we did not observe evidence of oxidative damage in response to our exercising protocols. We could conclude that the anti-inflammatory response observed is likely not caused by oxidative damage. It should, however, be noted that oxidative damage is a fine process that can occur in multiple cellular levels, and perhaps the techniques that we used might not be sensitive enough to detect it. Thus, future studies should investigate whether resistance training-inducing oxidative damage could result in an anti-inflammatory response as a compensatory mechanism.

In summary, our results suggest that, at equivalent workloads, an arm-based (bench press) exercise induced higher anti-inflammatory effects and muscle damage compared with a leg-based (half-squat) exercise. The molecular mechanisms inducing such an anti-inflammatory effect remain to be ascertained. We suggest that the higher mechanical stress observed in the arm-based exercise may induce a compensatory anti-inflammatory effect.

Authors' Contributions

JAV and JRH conceived the study, and participated in its design and coordination. JAV, CC, RAC and LF participated in all the measurements of the study. JRPD performed the statistical analysis. JAV and RAC wrote the manuscript. All authors have read and approved the final version of the manuscript, and agreed with the order of presentation of the authors.

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Competing Interests

None of the authors declare competing financial interests.

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FIGURE LEGENDS

Figure 1. Values of muscle damage markers in bench press (black bars) and half-squat (white bars) exercises in response to maximum incremental strength tests at different time-points. (A) Creatine kinase-MB (CK-MB). (B) Lactate dehydrogenase (LDH). (C) α-Actin. *p<0.05, bench press vs. half-squat. #p<0.05 as indicated in the figure. Well number 2 of the standard curve (0.4 µg) was used as a positive control.

Figure 2. Values of inflammatory response in bench press (black bars) and half-squat (white bars) exercises in response to maximum incremental strength tests at different time-points. (A) Interleukin 10 (IL10). (B) Interleukin 6 (IL6). (C) Tumor necrosis factor alpha (TNFα). *p<0.05, bench press vs. half-squat.