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2024-01-01

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### Recommended Citation

Carmona, G., Moras, G., Bescós, R., García-Fresneda, A., & Cadefau, J. (2024) 'Selective fast fiber damage after leg press exercise leading to failure: a pole vaulter case report', *Retos*, 51, pp. 1375-1380. Available at: <https://doi.org/10.47197/RETOS.V51.98084>

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**Published in:**

Retos

**DOI:**

[10.47197/RETOS.V51.98084](https://doi.org/10.47197/RETOS.V51.98084)

**Publication date:**

2024

**Document version:**

Publisher's PDF, also known as Version of record

**Link:**

[Link to publication in PEARL](#)

**Citation for published version (APA):**

Carmona, G., Moras, G., Bescós, R., García-Fresneda, A., & Cadefau, J. A. (2024). Selective fast fiber damage after leg press exercise leading to failure: a pole vaulter case report. *Retos*, 51, 1375-1380. <https://doi.org/10.47197/RETOS.V51.98084>

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## Selective fast fiber damage after leg press exercise leading to failure: a pole vaulter case report Daño selectivo de fibras rápidas tras un ejercicio en prensa de piernas hasta al fallo volitivo: estudio de caso de un saltador de pértiga

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**Abstract.** The aim of this study was to investigate, in a trained pole vaulter (PV) and in an endurance-trained physical education student (PE), the effect of a leg press exercise leading to failure (LPF) on changes in serum activity of muscle enzymes and serum concentration of fast (FM) and slow (SM) myosin isoforms, while simultaneously examining mechanical output components as indicators of performance and fatigue developed throughout exercise. A case report study design based on an observational comparison of response between two dichotomous participants, PV and PE, was used. Differences between the participants' exercise outputs were examined by unpaired *t*-test or Mann-Whitney test and serum levels of muscle enzymes and myosin isoforms were analyzed at baseline and 24 and 48 hours after LPF. Exercise output analyses showed that the PV's average fatigue index was significantly higher ( $P = 0.004$ ). Moreover, during the first six sets, the concentric average power exerted by the PV was significantly ( $P < 0.01$ ) higher (range: 14% to 35%) than that of the PE. The PV only showed acute mild increases of serum creatine kinase (CK) and FM 24 hours after exercise. In contrast, the PE presented persistent serum rises of several muscle enzymes and SM until 48 h after exercise. The PV's exercise output revealed an explosive (power-oriented) profile leading to selective mild damage of fast fibers. In contrast, the PE exercise output showed a fatigue-resistant profile, which induced greater muscle enzyme activity and SM serum concentration, suggesting a higher extent of slow fiber damage.

**Keywords:** muscle damage, creatine kinase, myosin isoforms, power output.

**Resumen.** El objetivo de este estudio fue investigar, en un saltador de pértiga entrenado (PV) y en un estudiante de educación física entrenado en resistencia (PE), el efecto de un ejercicio de prensa de piernas hasta el fallo volitivo (LPF) sobre los niveles séricos de enzimas musculares e isoformas de la miosina rápida (FM) y lenta (SM), examinando simultáneamente las variables mecánicas como indicadores del rendimiento y la fatiga desarrollados a lo largo del ejercicio. Se utilizó un diseño de estudio de caso único basado en una comparación observacional de la respuesta entre dos participantes dicotómicos, PV y PE. Las diferencias entre los resultados del ejercicio de los participantes se examinaron mediante prueba-*t* para muestras independientes o el test de Mann-Whitney. Se analizaron los niveles séricos de enzimas musculares e isoformas de miosina al inicio y a las 24 y 48 horas después del LPF. Los análisis de los resultados del ejercicio mostraron que el índice medio de fatiga del PV fue significativamente mayor ( $P = 0,004$ ). Además, durante las seis primeras series, la potencia concéntrica media ejercida por el PV fue significativamente ( $P < 0,01$ ) mayor (rango: 14% a 35%) que la del PE. El PV sólo mostró leves aumentos agudos de la creatina quinasa (CK) sérica y de la FM 24 horas después del ejercicio. Por el contrario, el PE presentó aumentos séricos persistentes de varias enzimas musculares y SM hasta 48 h después del ejercicio. Las variables mecánicas del ejercicio del PV revelaron un perfil explosivo (orientado a la potencia) que conducía a un daño leve selectivo de las fibras rápidas. Por el contrario, las variables mecánicas del ejercicio del PE mostraron un perfil resistente a la fatiga, que indujo una mayor actividad enzimática muscular y concentración sérica de SM, sugiriendo un mayor grado de daño de las fibras lentas.

**Palabras clave:** daño muscular, creatina quinasa, isoformas de la miosina, producción de potencia.

Fecha recepción: 24-01-23. Fecha de aceptación: 16-11-23

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### Introduction

High-intensity leg press exercise leads to increases in indirect markers of muscle damage, such as creatine kinase (CK) (Kusnanik et al., 2023; Zakaria et al., 2023), range of motion (ROM) (Ayubi et al., 2023) and soreness in the knee extensor muscles (Zakaria et al., 2023). Moreover, although the physiological responses to exhausting leg press exercise leading to failure (LPF) have been well-documented in recreational endurance-trained athletes (Gorostiaga et al., 2012, 2014) with a high mean percentage of slow (type I) fibers ( $65 \pm 12\%$ ) (Gorostiaga et al., 2010, 2012), it is not possible to generalize such results to power athletes, who are expected to have higher proportions of fast (type II) fibers. Fast (type II) fibers generate high peak power and contract with high shortening speed, primarily determined by myosin isoforms (Schiaffino & Reggiani, 2011; Westerblad et al., 2010). Their energy

failure significantly decreases muscle power output, especially during fast movements (Sargeant, 2007). In this regard, LPF is primarily used in exercise training for muscle strength and hypertrophy (Burd et al., 2012; Kraemer et al., 1987). It induces selective fatigue of fast (type II) fibers and then progressive recruitment of slow (type I) fibers, which decreases mechanical efficiency in the final part of the exercise (Gorostiaga et al., 2010). Considering that exhaustive resistance training with a demanding eccentric component typically leads to mild or moderate muscle damage (Paulsen et al., 2012), it was hypothesized that LPF would result in indirect evidence of myofibrillar disruptions in both fast (type II) and slow (type I) fibers. However, the extent of damage to these fiber types induced by LPF remains uncertain, particularly when highly trained athletes, who are expected to have a higher proportion of fast (type II) fibers, such as pole vault athletes, are involved.

Therefore, the purpose of this study was to investigate

the effect of LPF on changes in serum activity of muscle enzymes and serum concentration of myosin isoforms, as novel indirect biomarkers of sarcomere disruptions of fast (type II) and slow (type I) fibers (Carmona et al., 2019; Carmona, Guerrero, et al., 2015; Carmona, Roca, et al., 2015) in a highly trained pole vaulter (PV). We simultaneously studied the mechanical output components as indicators of performance and fatigue developed during the exercise. Furthermore, the PV case study was compared to a control subject with similar characteristics to those of the samples described in previous studies involving exhausting leg press exercise models (Gorostiaga et al., 2010, 2012).

## Case report

### Study design

A case report study design based on an observational comparison of response between two dichotomous subjects was used. The independent variable in this experiment was a single bout of LPF performed by a PV (case subject) and an endurance-trained physical education student (PE) (control subject). The dependent variables assessed in the subjects were serum concentration of fast and slow myosin isoforms (FM and SM respectively) as indirect biomarkers of fiber-type-specific sarcomere damage (Carmona et al., 2019; Carmona, Guerrero, et al., 2015; Carmona, Roca, et al., 2015), and commonly used muscle damage biomarkers such as creatine kinase (CK) (Brancaccio et al., 2010).

### Subjects

Two participants were recruited for the study: a national level, under-23, pole PV (age 22.8 years, weight 73.8 kg, height 1.72 m) who trained ~12 hours per week, had six years of athletic training experience, and a personal best of 4.85 m; and a physical education student (PE) (age 22.5 years, weight 69.9 kg, height 1.71 m) who performed 6-7 hours per week of physical activity consisting of endurance running, but not trained for competition. Subjects had not suffered any myotendinous injury for 1-year before the experiments and were asked not to perform any exercise four days before and throughout the experimental period. These precautions were implemented to establish a consistent baseline for the assessment of muscle damage biochemical markers. The study conformed to the World Medical Association's code of ethics (declaration of Helsinki) and approval was given by the Ethics Committee of the Catalan Sports Council (*Generalitat de Catalunya*) (0099S/690/2013).

## Procedures

### Blood sampling and processing

Blood samples of 5 mL were collected from an antecubital vein before exercise (baseline) and 24 and 48 h after exercise. Samples were allowed to clot for 30 min and then centrifuged at  $3000 \times g$  for 10 min. Three aliquots of serum were obtained and stored at  $-80^{\circ}\text{C}$  until they were

analyzed for enzymatic activity or concentration, and myosin isoforms concentration.

Automatized analyses of CK, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were performed in an Advia 2400 (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA). Creatine kinase MB isoform (CK-MB) analyses were performed using a Dimension Clinical Chemistry System (Siemens Healthcare Diagnostics, Tarrytown, NY, USA), with an analytical measurement range of  $0.5\text{--}300 \text{ ng}\cdot\text{mL}^{-1}$ . The concentration of myosin isoforms, FM and SM, was measured using an enzyme-linked immunosorbent assay (ELISA sandwich) which is described elsewhere (Carmona, Guerrero, et al., 2015; Guerrero et al., 2019). Briefly, two plates (Corning 96-well EIA/RIA, Sigma Aldrich, Poole, UK) were coated overnight at  $4^{\circ}\text{C}$  with capture monoclonal antibodies (all Sigma Aldrich, Poole, UK), anti-myosin (skeletal, fast) clone My-32 and anti-myosin (skeletal, slow) clone NOQ7.5.4D, for FM and SM assessment respectively. The plates were then washed 3 times (phosphate buffered saline, pH 7.4, 10 mM) and blocked with block buffer (Super Blocking Buffer, Thermo Fisher Scientific Inc., Rockford, Illinois, USA) before being incubated (60 min at  $37^{\circ}\text{C}$ ). After a wash step, samples ( $10 \mu\text{L}$ ) were added by triplicate, and a calibration curve of 6-point serial dilution, from 0 to 250 ng, of commercial pure myosin of porcine muscle M0273 was obtained. To complete the ELISA, anti-myosin polyclonal antibody M7523 was used as the primary antibody, and mouse anti-IGG linked to peroxidase A6154 as the secondary antibody. Finally, myosin concentrations ( $\mu\text{g}\cdot\text{L}^{-1}$ ) were obtained by the interpolation of the calibration curve ( $r^2 > 0.95$ ). Intra-assay coefficients of variation were below 10.0% for both FM and SM. The linearity of the FM assay was 80% and 90% for SM.

### One repetition maximum assessment

After three warm-up sets consisting of approximately 10 repetitions with a low weight, participants were directed to execute a single maximum repetition on a pneumatic leg press machine (Air300, Keiser Corporation, Fresno, CA, USA). Participants started the test from a knee  $90^{\circ}$ -angle static position using the adjustable seat of the pneumatic machine, and performed a concentric extension to reach the full extension of  $180^{\circ}$  against the resistance. The resistance was gradually incremented until the one-repetition maximum (1RM) (Valencia Sánchez et al., 2023). Adjustments to the resistance between trials were made to minimize the total number of attempts necessary to achieve the 1RM. The range of lifts required to reach 1RM varied from three to six attempts (Wisloff et al., 2004).

### Exercise

Participants performed 9 sets of concentric-eccentric repetitions until volitional failure at a workload equivalent to 75% of concentric 1-RM in the pneumatic leg press,

which allows for constant resistance throughout the whole range of motion independently of the velocity of exercise (Escamilla et al., 2012). A 3-min period of passive recovery was interspersed between sets. The participants were encouraged to complete the whole range of motion of every repetition as rapidly as possible. The concentric and eccentric velocity and the concentric work and power of each repetition were recorded using a linear encoder sampled at a frequency of 100 Hz by MuscleLab 4020e (Ergotest Technology AS, Langesund, Norway) system (Carmona, Guerrero, et al., 2015). Fatigue index (%) (FI) was calculated as follows:

$$([Max\ Power - Min\ Power] / Max\ Power) \times 100$$

The participants were provided with visual feedback (MuscleLab software) and verbal encouragement in order to maximize power output and achieve muscle failure. It must be emphasized that the aim of the study was not to compare two equivolumic exercises.

### Statistical analyses

The normality of each variable was tested using the Shapiro-Wilk test. The unpaired *t*-test or Mann-Whitney test (the choice was dependent on a normality test for Gaussian distribution) were used to test differences between subjects' mechanical output variables. Friedman's test with post-hoc Wilcoxon signed-rank tests with a Bonferroni correction was used to test differences between the PV's average power output in the first set and the rest of the sets. Data are presented as mean  $\pm$  standard deviation. The level of significance was set at  $P < 0.05$ . All the statistical analyses were conducted using the SPSS version 20.0 (SPSS Statistics, IBM Corp., Armonk, New York, USA) statistical analysis software.

## Results

### Exercise work, velocity and power outputs

The 1-RM was 320 kg for the PV and 250 kg for the PE. The total repetitions performed were 132 ( $14.7 \pm 2.7$  average repetitions per set until failure) and 138 ( $15.0 \pm 5.1$  average repetitions per set until failure) for the PV and the PE respectively. The PV performed higher total work during exercise, but no average work differences were found between participants (Fig. 1[a]). The fatigue index was greater in the PV in every set, and significant differences were found between the participants' average fatigue index ( $P = 0.004$ ) (Fig. 1[b]). The PV applied higher concentric average velocity during the first sets of the LPF (Fig. 1[c]). Compared to the concentric average power exerted by the PV during the first set of the LPF, significant ( $P < 0.01$ ) reductions (range: -12% to -19%) were found during the exercise, with the exception of sets 2 and 6, in which no significant differences were observed. Moreover, during the first six sets the concentric average power exerted by the PV was significantly ( $P < 0.01$ ) higher (range: 14% to 35%) than that of the PE (Fig. 1[d]).

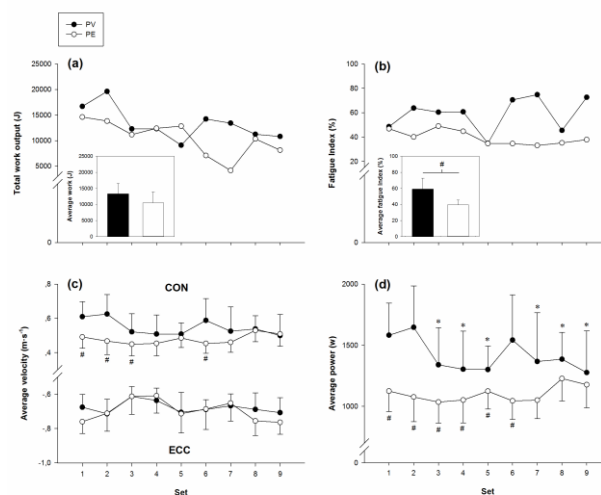


Figure 1. Leg press leading to failure output variables comparison between a pole vaulter (PV) and a physical education student (PE) at baseline and 24 and 48 h after exercise. Total work output (sum per set) and average work  $\pm$  standard deviation per set (a), fatigue index per set and average fatigue index  $\pm$  standard deviation of the whole exercise (b), average concentric (positive values) and eccentric (negative values) velocities  $\pm$  standard deviation per set (c), and average concentric power  $\pm$  standard deviation per set (d). CON, concentric. ECC, eccentric. #Significant difference between participants at  $P < 0.01$ . \*Significantly lower than the first set of values at  $P < 0.01$ .

### Biochemical markers of muscle damage

A clearly different response in biochemical markers was observed between participants. While, the PV showed slight serum increases in CK (from 183 to 405 IU·L<sup>-1</sup>) and CK-MB (from 0.5 to 1.6 ng·mL<sup>-1</sup>) at 24 h, and a clearly decreasing trend to enzyme baseline activity values at 48 h after exercise, the PE presented sharp serum rises, over the clinical normality range, of CK-MB (from 0.5 to 4.4 ng·mL<sup>-1</sup>) at 24 h, and of CK (from 142 to 1000 IU·L<sup>-1</sup>) and AST (from 24 to 41 IU·L<sup>-1</sup>) at 48 h after exercise (Fig. 2). The PV only showed mild increases in serum FM (from 1557 to 1998  $\mu$ g·L<sup>-1</sup>) at 24 h and remained high (1928  $\mu$ g·L<sup>-1</sup>) 48 h after exercise. In contrast, the PE presented moderate serum rises of SM until a peak (from 1303 to 1892  $\mu$ g·L<sup>-1</sup>) 48 h after exercise (Fig. 3).

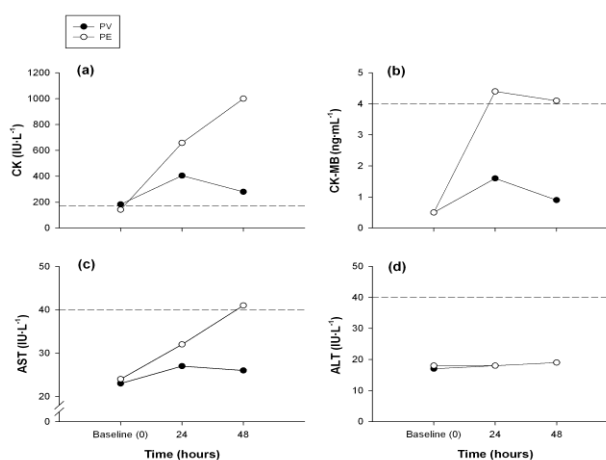


Figure 2. Muscle enzyme serum activity comparison between a pole vaulter (PV) and a physical education student (PE) at baseline and 24 and 48 h after exercise. Creatine kinase (CK) (a), creatine kinase MB isoform (CK-MB), (b), aspartate aminotransferase (AST) (c), and alanine aminotransferase (ALT) (d). A dashed line indicates the upper limit of clinical normality values.

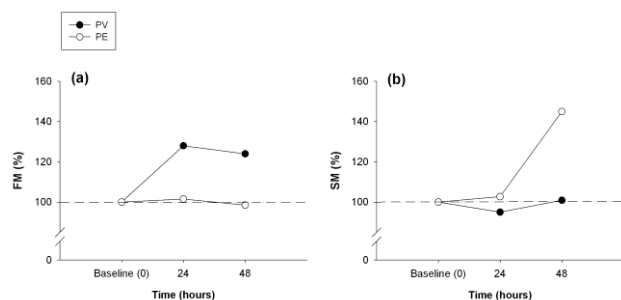


Figure 3. Fiber-type-specific sarcomere proteins' serum concentration comparison between the pole vaulter (PV) and physical education student (PE) at baseline and 24 and 48 h after exercise. Fast myosin (FM), and slow myosin (SM). Data are normalized to baseline values.

## Discussion

Results revealed that the PV's exercise work and power outputs were in line with an explosive (power-oriented) profile, leading to selective, mild damage of fast (type II) fibers. In contrast, the PE exercise work and power outputs indicated a fatigue-resistant profile, which produced greater muscle enzyme activity and SM serum concentration, indicating a greater extent of slow (type I) fiber damage.

This case report study presents unique and novel data from a highly trained national level PV. Pole vault competitors have similar characteristics to sprint athletes, since a high approach speed is necessary in this track and field event (Frere et al., 2010; Frère et al., 2017; Gross et al., 2020; Liu et al., 2011) so a high percentage of fast (type II) fibers is expected in these athletes. Unfortunately, to the best of our knowledge, there are no previous histochemical studies involving trained pole vault athletes. Probably, the difficult access and recruitment of these athletes can explain this lack of data. Data in elite sprint athletes is also limited, but Korhonen et al. (Korhonen et al., 2006) stated that young sprint trained competitors (18-33 years) present a high relative fast-fiber percentage in the vastus lateralis area ( $59 \pm 6\%$ ). Despite the anticipation of a higher percentage of fast (type II) fibers in pole vaulters (PV) due to the high-speed requirements in this track and field event (Gross et al., 2020), our hypothesis was that indirect evidence of myofibrillar disruptions in both fast (type II) and slow (type I) fibers could be observed following LPF. Contrary to our hypothesis, results indicate that selective, mild, fast-fiber damage was induced following LPF in the case of the PV.

### Exercise work, velocity and power outputs

During the first two sets of LPF, the PV developed a high average power and velocity, which are related to the fast-fiber capacity to generate great power output and contract with elevated shortening speed (Sargeant, 2007; Van Vossel et al., 2023). LPF required a maximal effort from the PV, as reflected by a clear decrease in power output, observed from the third set onwards, which indicates energy failure and selective fatigue of fast (type II) fibers (Gorostiaga et al., 2010). Evident reductions in total work output per set also suggested progressive recruitment of slow (type I) fibers, with a

decrease in mechanical efficiency (Gorostiaga et al., 2010). In contrast, the PE showed a significantly lower power output, but an extraordinary capacity for maintaining its mechanical power throughout exercise, which could be related to slow (type I) fibers' specialization for fatigue-resistant response during continuous activity (Schiaffino & Reggiani, 2011). Although fatigue was not as evident as in the PV, a marked reduction in mechanical work probably reflected greater recruitment and progressive decrease in slow (type I) fibers' efficiency.

### Biochemical markers of muscle damage

Interestingly, the exercise power output profile was in accordance with the serum biochemical response of both participants. In the PV, selective recruitment and fatigue of fast (type II) fibers led to damage of those fibers, which was suggested by FM increases observed 24 h after LPF. Slight FM increases in serum have been previously related to mild exercise-induced muscle damage (few myofibrillar disruptions) (Carmona, Guerrero, et al., 2015). In contrast, the PE moderate serum SM increases after exercise, which suggested slow-fiber damage, probably related to higher recruitment and fatigue of these types of fibers during exercise (Carmona et al., 2019; Carmona, Roca, et al., 2015). Both FM and SM serum levels were high 48 h after LPF because of myosin complex degradation metabolism (Eble et al., 1999; Goll et al., 2008). The PV's slight FM increases in serum were accompanied by marginal CK activity rises at 24 h, returning to almost baseline values at 48 h after LPF, which is indirect evidence of a metabolic recovery status (Bessa et al., 2013), probably associated to an enhanced clearance capacity related to training adaptations (Baird et al., 2012; Pan et al., 2023). In contrast, the PE showed clinically relevant increases in CK, CK-MB and AST until 48 h after exercise, which suggests a greater extent of muscle damage. Moreover, sharp serum increases in CK-MB and AST activities reinforced the notion that slow (type I) fibers were damaged, since these enzyme activities are higher in slow (type I) fibers (Schantz & Henriksson, 1987; Yamashita & Yoshioka, 1991).

## Conclusion

Biochemical response seems to be closely related to exercise work and power outputs. We can conclude that the PV's exercise work and power outputs revealed an explosive (power-oriented) profile, leading to selective, mild damage of fast (type II) fibers. In contrast, the PE exercise work and power outputs showed a fatigue-resistant profile, which induced greater muscle enzyme activity and SM serum concentration, and suggests a greater extent of slow (type I) fiber damage.

### Conflicts of interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

## Limitations

The limitations are those inherent to case report studies, such as lack of ability to generalize and danger of overinterpretation. To contrast the results from the present work, future studies should involve larger samples of subjects with high percentages of fast (type II) fibres.

## Acknowledgements

The authors would like to thank the athlete and the physical education student who gave their permission and were positive about the publication of this data.

## Authors' contributions

Gerard Carmona, Gerard Moras, Raul Bescós, Adrián García-Fresneda and Joan Aureli Cadefau conceived and planned the experiments. Gerard Carmona, Gerard Moras and Raul Bescós performed the experiments. Gerard Carmona and Gerard Moras processed the experimental data. Gerard Carmona analyzed the data, drafted the manuscript and produced the figures.

All authors read and approved the final version of the manuscript.

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