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ORIGINAL ARTICLE

Effects of essential amino acids supplementation on muscle damage following a heavy-load eccentric training session

Effets d'une complémentation en acides aminés essentiels sur les microlésions musculaires suite à une séance d'entraînement excentrique à charge lourde

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KEYWORDS

Essential amino acids supplementation;
Eccentric exercise;
Delayed-onset muscular soreness;
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Myoglobin

Summary

Aim. – Unaccustomed physical exercise, particularly repeated eccentric muscle contractions, induces muscle soreness and alterations on muscle cellular structure. An increase in myofibrillar protein accretion can occur in the early post-exercise period and be potentiated by essential amino acid ingestion. We hypothesized that essential amino acid supplementation could reduce the efflux of indirect markers of muscle damage and delay the onset of muscular soreness in the week following a heavy-load eccentric training session.

Methods. – Twenty-three randomly assigned young males performed a bench press exercise under eccentric condition. They were subdivided into a placebo group ($n=11$) and an essential amino acids group ($n=12$). The effect of the training session was assessed by analysing two indirect markers of muscle damage, namely plasma concentrations of creatine kinase and myoglobin measured before, immediately after, and post-workout day 1, 2, 3, 4 and 7. Muscle soreness was evaluated by a visual analogy scale at the same time point as the markers of muscle damage.

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MOTS CLÉS

Acides aminés
essentiels ;
Exercice
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Douleurs musculaires
d'apparition
retardée ;
Créatine kinase ;
Myoglobine

Results. – The training session induced a significant increase in muscle soreness in both placebo and essential amino acids groups. Plasma creatine kinase release increased significantly at D+3 and D+4 while myoglobin efflux rose at D+3 in placebo group only. No statistical differences were observed between groups for the two indirect markers of muscle damage. Gaussian distribution was found to be the best-fit model for the plasma myoglobine and creatine kinase concentration curves. F-test support that individual curves were statistically distinguishable as comparing to the best-fit values of the three parameters (area, SD and mean) between placebo and essential amino acids group data sets ($P < 0.01$).

Conclusion. – These data indicate that essential amino acids supplementation do have minor effects on the overall plasma release of indirect markers of muscle damage during the recovery period without impact on delayed-onset muscular soreness.

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Résumé

Objectifs. – L'exercice physique inhabituel, en particulier les contractions musculaires excentriques répétées, engendre des douleurs musculaires et des altérations de la structure des fibres musculaires. Une augmentation de la synthèse de protéines myofibrillaires peut survenir au début de la période post-exercice et être potentialisée par une ingestion d'acides aminés essentiels. Nous avons émis l'hypothèse qu'une complémentation en acides aminés essentiels pourrait réduire la production de témoins de la lyse musculaire et retarder l'apparition de douleurs musculaires dans la semaine suivant une séance d'entraînement excentrique à charge lourde.

Méthodes. – Vingt-trois jeunes hommes ont effectué un exercice de développé couché guidé en modalité excentrique. Ils ont été subdivisés, de manière aléatoire, en un groupe placebo ($n = 11$) et un groupe acides aminés essentiels ($n = 12$). L'effet de la séance d'entraînement a été évalué en analysant deux marqueurs indirects de lésions musculaires, à savoir les concentrations plasmatiques de créatine kinase et de myoglobine, mesurées avant, immédiatement après la séance d'entraînement et les jours 1, 2, 3, 4 et 7 post séance. La douleur musculaire a été évaluée à l'aide d'une échelle analogie visuelle au même moment que les marqueurs des lésions musculaires.

Résultats. – La séance d'entraînement a provoqué une augmentation significative des douleurs musculaires dans les groupes placebo et acides aminés. La libération de créatine kinase plasmatique a augmenté significativement à j+3 et j+4 tandis que le flux de myoglobine a augmenté à j+3 dans le groupe placebo uniquement. Aucune différence statistique n'a été observée entre les groupes pour les deux marqueurs indirects de lésions musculaires. La distribution gaussienne s'est avérée être le modèle le mieux adapté aux courbes de concentration de la myoglobine et de la créatine kinase dans le plasma. Le test F confirme que les courbes individuelles étaient statistiquement différentes entre l'ensemble des données des groupes placebo et acides aminés essentiels ($p < 0,01$) comparées aux valeurs les mieux ajustées des trois paramètres (superficie, écart-type et moyenne).

Conclusion. – Ces données confirment que la complémentation en acides aminés essentiels pourrait avoir des effets mineurs quant à la libération plasmatique globale des marqueurs indirects des lésions musculaires au cours de la période de récupération sans toutefois impacter l'apparition de douleurs musculaires retardée.

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1. Introduction

Eccentric contractions refer to the lengthening of active muscle fibres, producing greater maximal muscle tensions as compared to isometric or concentric contractions [1–3]. Unaccustomed eccentric exercise is likely to induce muscle damage as evidenced by delayed-onset of muscle soreness (DOMS), loss of muscle strength, decrease in range of motion, histological disturbance of muscle and connective tissue and muscle swelling due to subsequent inflammatory response [4].

DOMS is characterized by dull aching pain in the skeletal muscles, within the first 24 hours, peaks 1–3 days after exercise and those symptoms typically disappear after 7–10 days [5,6]. Accordingly, increased muscle soreness, in combination with loss of muscle strength, may have a detrimental effect on muscle function. However, light exercise, stretching, therapeutic massage, cryotherapy, partial immobilization, electrical stimulation and anti-inflammatory drugs did not reveal DOMS reduction [7–9].

Mechanical myofibrillar disruptions are thought to occur during intensive eccentric contractions [10,11], leading to

protein degradation of muscle fibre. Although inflammation and degradation of damaged tissue also stimulate protein synthesis [12,13], the net protein turnover status is mostly determined by catabolic events during post-exercise recovery [14,15]. Therefore, nutritional strategies could be advantageous to alleviate the deleterious effects of eccentric exercise. Indeed, it is well known that protein or amino acids supplementation stimulates muscle protein synthesis and that exercise potentiates the induced protein synthesis stimulation [16–19]. Moreover, a combined essential amino acids (EAA) and carbohydrate supplementation reduces the effect of strength-training exercise on myofibrillar protein breakdown and may favour the maintenance of myofibrillar protein [20]. Thus, EAA supply seems to be a key factor in regulating protein synthesis and degradation after strength exercise [15,16,18].

Although it is generally accepted that EAA supply induces an increase in protein synthesis and has positive effects on the net protein balance, it is unclear if such an intervention could affect the recovery of disrupted myofibres by eccentric exercise. Since structural damage of muscle fibres is accompanied by the leakage of proteins such as creatine kinase (CK) and myoglobin (Mb) from the muscle fibres to the blood circulation, EAA supplementation, administered during the post-exercise recovery period, could reduce the release of these markers of muscle sarcolemma damage while attenuating muscle soreness.

We hypothesized that EAA supplementation, ingested immediately after and during the recovery period following a heavy-load eccentric session would attenuate muscle soreness and the release of CK and Mb.

2. Methods

2.1. Subjects

Twenty-three healthy Caucasian males (25.6 ± 4.6 years) participated in the study. They were informed about the experimental procedures, and written consent was obtained. Subjects were recruited on the University campus and were recreationally active students in physical education or physiotherapy. They were not engaged in any specific strength-training programme and did not experience eccentric exercise for at least six months prior to the start of the study. They were instructed to refrain from any physical activity and to avoid any action aimed at reducing muscle soreness (therapeutic massage, stretching exercise, anti-inflammatory drug intake) throughout the duration of the study. Subjects were matched for strength and nutritional protein intake and were then randomly assigned to one of the two groups: the first one received an essential amino acids supplementation combined with sucrose (EAA, $n = 12$) while the second group received an isocaloric placebo supplementation (PLA, $n = 11$). The experimental protocol was approved by the local University Hospital Ethics Committee.

2.2. Nutritional supplementation

Both PLA and EAA groups received one daily dietary supplementation at the same time of the day. EAA supplementation was a flavoured 30 g mixture in powder form composed by

15 g of EAA and 15 g of sucrose. Proportions in EAA were 11% of histidine, 10% of isoleucine, 19% of leucine, 15% of lysine, 3% of methionine, 15% of phenylalanine, 15% of threonine and 12% of valine. PLA supplementation contained 30 g of sucrose and the same artificial sweetener as for the EAA supplement. Both supplements were dissolved in 200 mL water and consumed immediately after completing the eccentric training session on the first day of the study and at the same time during each 6 days following the exercise session. PLA and EAA supplement formula added respectively an extra 119 kcal and 120 kcal, to subject's diet each day. Supplements were provided by Hedelab Company, Belgium. Supplements were labelled by a number without subjects knowing to which composition it corresponded and drank each day in the presence of an investigator, to assure subject's 100% compliance to the supplementation.

2.3. Dietary intakes

Before the start of the study, dietary intake was assessed by a food questionnaire over 7 continuous days. The participants were clearly instructed to maintain their normal eating pattern and to report detailed information about each food item as accurately as possible considering preparation and composition of foods and portion size. For the latter, they were asked to weigh the items before cooking using their personal weigh-scale. When this was not possible, food quantities were estimated using food models. The subjects returned the completed food records before the start of the study. All subjects were questioned individually by a single investigator, who reviewed food record details with the subject upon completion. Diets were analysed by Nutrilog Software (Nutrilog version 2.6, France). Specific information (ingredients and amounts) on local foods had been added to the database (Nubel, 4th edition). Before training, baseline value for protein intake per kg of body mass was $1.22 \pm 0.17 \text{ g}\cdot\text{kg}^{-1}$ for the PLA group and $1.21 \pm 0.12 \text{ g}\cdot\text{kg}^{-1}$ for the EAA group. No statistical difference was observed between both groups ($P = 0.93$).

2.4. Strength evaluation

Approximately one week before the start of the study, subject's maximal strength was assessed by determining the one repetition maximum (1 RM) for bench press exercise with guided bar and weighted plates. Warm-up consisted of 3 sets with a light load that permitted at least 10 repetitions. 1 minute of rest was provided after each set. Subjects thereafter performed 1–3 repetitions at 80–85% of perceived maximum. The workload was then enhanced by 2.5 kg until subjects failed to complete an attempt properly. Two to three minutes of rest was provided between attempts.

2.5. Eccentric exercise

Subjects performed a session of bench press exercise performed in eccentric condition on the first day of the study. The training session was performed at the University's sport facilities. The eccentric session consisted of 8 sets of 6 repetitions of bench press exercise performed in a guided

device. Subjects laid in supine position with bent legs and feet placed on the bench to stabilize the lower back. The movement started with the elbow almost fully extended and was completed when hands and elbows were placed in line with the processus xiphoideus. Subjects were told to lower the load over the range of movement in 4 seconds following the investigator's counting from 0 to 4. Subjects tried to keep the velocity as constant as possible (~10 cm/s) throughout the range of motion and the investigator used a metronome to assure accurate counting. After each eccentric action, investigators returned the bar to the starting position so that subjects performed no concentric action with the load. Approximately 3 seconds rest were provided between each eccentric action, while 2 minutes rest were accorded between sets. The firsts sets of the bench press exercise were performed at 110% of the subject's 1 RM. If participants were not able to complete all repetitions of a given set in 4s, the workload was reduced for the next set in order to complete the whole session. The training sessions were supervised by a graduate in physical education and a graduate in sport physiotherapist to assure proper technique and exercise intensity adherence.

2.6. Criterion measures

2.6.1. Muscle soreness

Muscle soreness was evaluated during palpation by a visual analogy scale (VAS) [21] consisting of a 10 cm line with "no pain" on the left end and "extremely painful" on the right end. Subjects placed a marker along the line representing the perceived soreness felt in the triceps and pectoral muscles. Measurements were taken at 1, 2, 3, 4 and 7 days after the completion of the eccentric exercise session.

2.6.2. Blood analyses

Approximately 5 mL of blood was drawn from the ante-cubital vein at all measurement time and centrifuged for 15 minutes at 3500 rpm at 4°C. Plasma concentrations of CK and Mb were used to estimate muscle damage. These markers used in several previous studies [22,23] were measured before, immediately after and up to 7 days after the eccentric exercise. Plasma were extracted and stored at -20°C until CK activity and Mb concentration analyses. Plasma CK activity was determined spectrophotometrically using a Microparticle Enzyme Immunoassay (Model Abbott AXSYM System). Plasma Mb concentration was analysed using a particle-enhanced immunoturbidimetry (Dade Behring TurbiTimer System, Siemens).

2.7. Statistical analysis

Statistical tests were performed with Graphpad prism software (version 4.0, San Diego, California, USA). Descriptive statistics, including means, standard deviations (SDs) and standard errors (SEs) were calculated for each parameter. The data is presented as means ± SD in the text and as means ± SE in the figures. The decline in workload between the sets during the eccentric training session and the changes in VAS, CK and Mb concentrations were analysed by means of two-way ANOVAs with repeated measures on two factors (time points × groups). When significant main effects

were observed, a Dunnett post-hoc test was used to identify the significant differences among means. Mb concentration and CK concentration distribution curves were generated using GraphPad Prism 4. Gaussian distribution was found to be the best-fit model for the tested compounds. Area, SD and mean were calculated using the equations for Gaussian distribution. Comparisons between different hierarchical models were quantified by an *F* test as, for example in our study, in the case of a single Gaussian versus the sum of two Gaussians. The equation used for a single Gaussian was $y = Area / (SD * (2 * \pi)^{0.5}) * \exp(-0.5 * ((x - Mean) / SD)^2)$. The *F* value can be used to quantify the improvement in fit achieved by a more complex model compared with a simpler one. The *F* value is calculated as the relative increase in the sum of squares divided by the relative increase in degrees of freedom, going from the more complicated to the simpler model. The best-fit value of three parameters was compared between placebo and EAA group data sets: the area, the SD and the mean, in order to test whether one curve suffices for all data sets or whether the individual curves are statistically distinguishable. All of these objective criteria of evaluation were in full agreement with the visual impression of the fits. Regression lines were provided using GraphPad Prism 4. For all analyses, the level of significance was set at *P* < 0.05.

3. Results

3.1. Workload

Only 2 subjects of each groups were able to complete the 8 sets of the eccentric exercise during the training session at a workload of ~110% of 1 RM. PLA and EAA groups performed the first set of the bench press exercise at a workload of 111 ± 2%. It was significantly reduced at the sixth set for the PLA group (100 ± 10% of 1 RM; *P* < 0.05), and at the seventh set for the EAA group (98 ± 13% of 1 RM; *P* < 0.01). The last set was performed at a mean load of 90 ± 18% and 96 ± 14% of 1RM, respectively for PLA and EAA groups (*P* < 0.001). No significant difference was found between groups (Fig. 1).

3.2. Muscle soreness

Consequent to the eccentric exercise, muscle soreness increased at day 1 post-exercise (D+1), peaked at D+2 (respectively 4.8 ± 1.9 and 4.1 ± 2.0 for the PLA and the EAA groups, *P* < 0.001) and remained increased at D+4 only in the PLA group (1.6 ± 1.0; *P* < 0.05). No intergroup differences were observed for VAS score (Fig. 2).

3.3. Changes in markers of muscle damage

Initial mean values of plasma CK concentrations were 202 ± 132 IU/L and 200 ± 122 IU/L, respectively for PLA and EAA groups. Those values were slightly above normal distribution according to the chemical laboratory (< 170 IU/L). Consecutive to the eccentric exercise, plasma CK release increased and reached significance at D+3 in PLA group only (10,929 ± 16,524 IU/L; *P* < 0.05) and was still enhanced at D+4, reaching values of 11,691 ± 17,050 IU/L

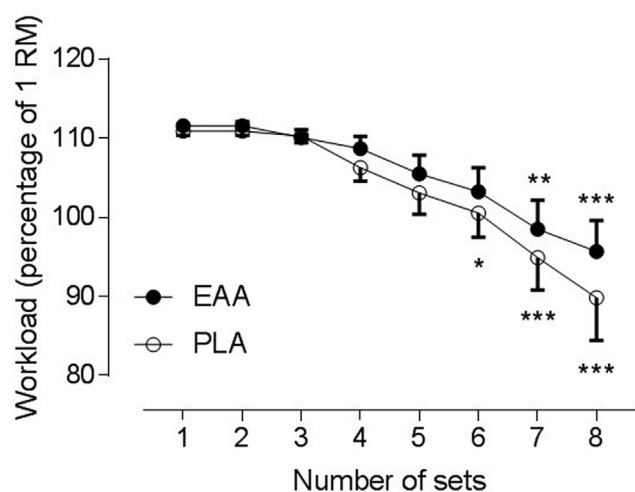


Figure 1 Decrease in workload expressed in percentage of 1RM during the eccentric exercise session. * denotes significant difference between the initial workload and the values of the following sets ($P < 0.05$). ** denotes significant difference between the initial workload and the values of the following sets ($P < 0.01$). *** denotes significant difference between the initial workload and the values of the following sets ($P < 0.001$).

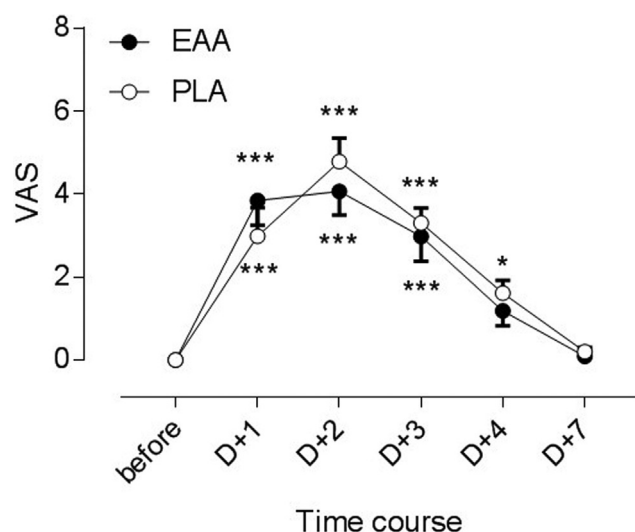


Figure 2 Increase in muscle soreness in response to an eccentric exercise. * denotes significant difference between the initial value and the values the days following the eccentric exercise ($P < 0.05$). *** denotes significant difference between the initial value and the values the days following the eccentric exercise ($P < 0.001$).

($P < 0.05$). CK concentrations recovered initial values at D+7 (3265 ± 4284 IU/L and 1137 ± 1037 IU/L, respectively for the PLA and the EAA group).

Initial mean values of plasma Mb concentrations were 29 ± 4 mg/L and 25 ± 0 mg/L, respectively for the PLA and the EAA group. Those values were also within the normal distribution according to the chemical laboratory (< 70 mg/L). Consecutive to the eccentric exercise, plasma Mb release increased and reached significance at D+3 in PLA group (962 ± 1397 mg/L; $P < 0.05$). At D+7, PLA and EAA groups

displayed plasma Mb concentrations of 160 ± 153 mg/L and 118 ± 195 mg/L (Fig. 3).

Gaussian distribution was found to be the best-fit model for plasma CK and Mb concentration curves. *F* test showed that individual curves were statistically distinguishable when comparing the best-fit values of the three parameters (area, SD and mean) between placebo and EAA group data sets ($P < 0.01$). PLA and the EAA CK concentration curves were respectively best fitted by following equations: $y = 53350 / (1.71 * (2 * \pi)^{0.5}) * \exp(-0.5 * (x - 4.00) / 1.71)^2$ ($r^2 = 0.62$), respectively $y = 21538 / (0.92 * (2 * \pi)^{0.5}) * \exp(-0.5 * (x - 3.48) / 0.92)^2$ ($r^2 = 0.66$). PLA and the EAA Mb concentration curves were respectively best fitted by following equations: $y = 2648 / (1.09 * (2 * \pi)^{0.5}) * \exp(-0.5 * (x - 3.04) / 1.09)^2$ ($r^2 = 0.63$), and $y = 2004 / (1.66 * (2 * \pi)^{0.5}) * \exp(-0.5 * (x - 3.52) / 1.66)^2$ ($r^2 = 0.33$) (Fig. 4).

Positive linear regression was found between the decrease in workload in percentage at the 8th set of the eccentric exercise and peak plasma CK release (IU/L) and peak Mb release (mg/L). The best-fit linear regression was $y = 447x + 3736$ ($r^2 = 0.20$; $P < 0.05$) for the plasma CK release, respectively $y = 37x - 164$ ($r^2 = 0.19$; $P < 0.05$) for the plasma Mb release (Fig. 5).

4. Discussion

The bench press exercise performed in eccentric condition elicited DOMS of the upper limb and a high response of the markers of muscle damage together with a strong individual variability. EAA ingestion during the week following eccentric training did not reduce muscle soreness or attenuate plasmatic CK and Mb release at any given point of time.

4.1. Impact of eccentric exercise

Delayed and prolonged muscle soreness accompanies unaccustomed eccentric exercise. Fridén et al. [13] observed ultrastructural myofibrillar disruptions, especially in regard to Z-bands, in subjects suffering from pronounced exercise-induced delayed muscle soreness. However, the underlying mechanisms of DOMS are not clearly understood and it is still debatable if myofiber damage is linked to DOMS. Indeed, although DOMS is a consequence of eccentric exercise-induced muscle damage, DOMS appears 24h after the exercise bout and peaks at 72h, while muscle alteration is detected on muscle biopsies immediately after the eccentric bout [5]. A recent review [24] suggests that DOMS may be induced by inflammation in the extracellular matrix, rather than myofiber damage. Indeed, muscle extracellular matrix proteins respond to eccentric exercise within hours and extracellular matrix remodelling may play an important part in protecting muscle against reinjury [25]. In our study, muscle soreness increased at D+1 and peaked at D+2 for both groups. VAS score returned to basal values, respectively at D+4 in the EAA group and D+7 in the PLA group. The time course of muscle soreness did not match the time course of the markers in muscle damage and we did not find any correlation between the magnitude of DOMS and the increase in markers of muscle damage. These data are consistent with

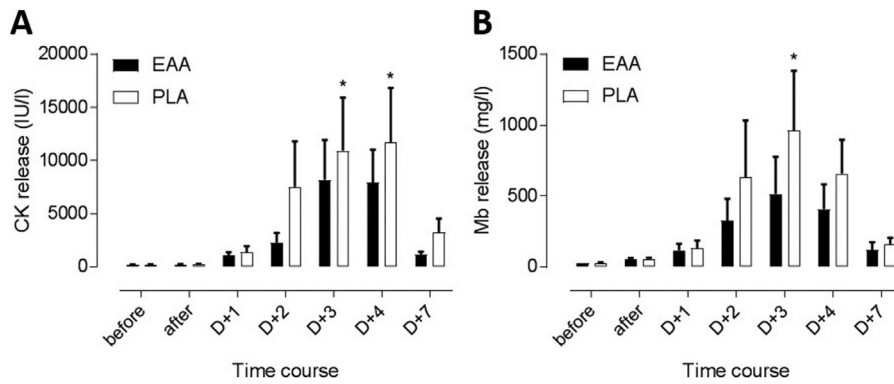


Figure 3 Increase in CK (A) and Mb (B) plasma release for PLA and EAA groups in response to an eccentric exercise. * denotes significant difference between the initial value and the values the days following the eccentric exercise ($P < 0.05$).

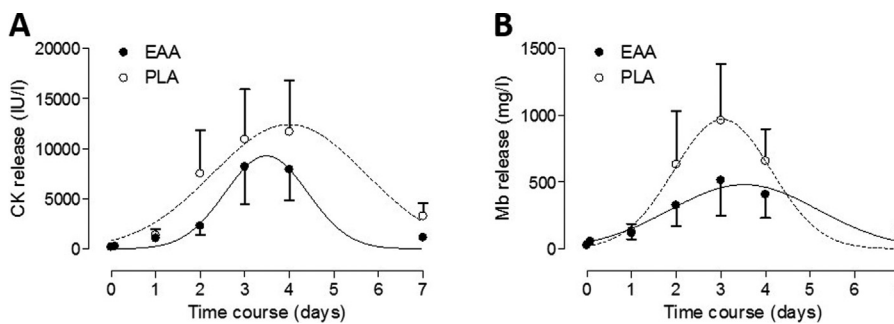


Figure 4 Gaussian relations between time course of the recovery and increase in CK (A) and Mb (B) plasma release following an eccentric exercise for PLA (○) and EAA (●). The curves were significantly distinguishable between both groups ($P < 0.01$).

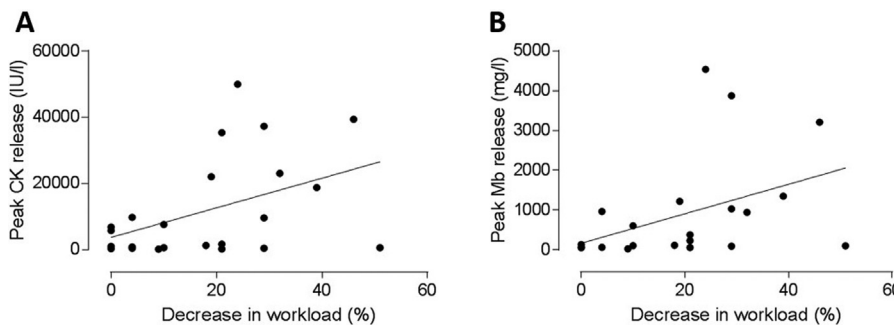


Figure 5 Relation between decrease in workload in percentage of initial load and respectively peak CK release in IU/L ($y = 447x + 3736$; $r^2 = 0.20$; $P < 0.05$) (A), peak Mb release in mg/L ($y = 37x - 164$; $r^2 = 0.19$; $P < 0.05$) during recovery.

the study of Nosaka et al. [26], concluding that DOMS is not an indication for the extent of muscle damage.

Unaccustomed eccentric contractions induce an increase in membrane permeability and subsequent leakage of muscle-specific proteins [27]. Mb and CK are widely used to quantify muscle damage, because they are responsive markers showing large variations from baseline. However, Mb and particularly CK release in blood serum after eccentric exercise have a high individual biological variability. In our study, peak Mb and CK release ranged from 57–4540 $\mu\text{g}/\text{mL}$ and 215–49,996 IU/L, respectively. Chen et al. [21] also

observed a very large individual variability after 30 eccentric contractions at 80% of pre-exercise maximal isometric force of the elbow flexors and they classified the subjects in four different groups depending on CK release magnitude. The high responder group achieved an average CK concentration of about 25,000 IU/L 4 days after the eccentric contractions. In our study, the workload intensity consisted of 110% of pre-exercise maximal isometric force and subjects performed in total 48 eccentric contractions. A greater mechanical strain associated to high-intensity muscle contractions and a higher number of contractions cause most likely greater

damage to contractile proteins and extracellular matrix [24], thus providing a possible explanation to the even higher CK values observed in our study as compared to the one of Chen et al. [21]. Damas et al. [28] observed similar individual peak values of CK activity following 30 maximum elbow flexors eccentric actions. Finally, the average workload had to be reduced by 16% and 21%, respectively in the EAA and PLA groups in the last set of the session in order to enable the subjects to complete the required number of repetitions at the imposed pace of 4 seconds. Indeed, Allen et al. [29] proposed that mechanical strain during eccentric exercise lead to “popped sarcomeres”, inducing in turn a reduced force. Actually, due to length heterogeneity, some individual sarcomeres elongating beyond filament overlap may fail to re-interdigitate after repeated eccentric contractions and become damaged, causing alterations of the sarcolemma and t-tubules structures [29]. As the region of damage propagates longitudinally and laterally [29], the high repetition number in our study probably gradually involved many sarcomeres. A new three-filament sarcomere model including titin was introduced by Herzog [30], suggesting that during eccentric contraction, titin increases its stiffness by binding calcium and force by attaching to actin upon muscle activation and thereby decreasing titin free spring length in the I-band region. Titin binding to actin at short initial sarcomere length, as it is the case in our study, the reduced free spring length of titin might cause Ig domain unfolding at much shorter length [30]. It is likely that the unfolding of Ig domains induced a great hysteresis in the force elongation curve of titin, and the decrease in workload was therefore associated with a great loss of the protein potential energy [30], putting myofibers in a more vulnerable state to muscle damage.

No correlation was found between the magnitude of muscle damage markers release and initial muscle strength, indicating that the individual biological variability of the muscle damage markers response did not depend on the strength level of the subjects. As none of our subjects was involved in a regular strength-training program before the study, the repeated bout effect probably did not influence the responsiveness of the subjects. We found a positive linear correlation between peak Mb or CK concentrations and the decrease in workload during the last set of the training session. Our results are in accordance with the study of Hody et al. [31] reporting a relationship between the decline in muscle work during 90 isokinetic maximal eccentric contractions of the quadriceps and next day CK response, but no association between the total work of the eccentric exercise and changes in muscle damage markers. In a study with a large sample and high variability of responses in muscle damage markers, Damas et al. [28] found a good correlation between the decline in MVC force immediately after 30 maximum elbow flexor eccentric actions and the largest MVC force loss in the days following the eccentric bout, suggesting that effort exerted during the eccentric exercise should be taken into account regarding an individual’s susceptibility to exercise-induced muscle damage. Muscle effort incurred by the eccentric exercise seems to affect the release of markers of muscle damage and to be related to the amount of muscle damage.

4.2. AAE vs. PLA

Following eccentric exercise, the induced mechanical alterations and metabolic stress associated with muscle damage stimulate intracellular interactions, which initiate subsequent tissue repair and remodelling [24].

Newham et al. [10] confirmed the occurrence of myofibrillar disturbances after eccentric contractions and observed greater and more widespread cross-striated band damage 24–48 h in comparison to immediately after exercise, suggesting an ongoing process of muscle repair [32]. Accordingly, unaccustomed high-intensity eccentric exercise increases the rate of myofibrillar protein turnover [32], resulting in an enhanced protein breakdown up to 10 days [33]. EAA stimulate protein anabolism by increasing muscle protein synthesis and exercise potentiates the effects of such an appropriated supplementation [34,35]. Thus, following EAA ingestion, muscle protein synthesis rates exceed muscle protein breakdown rates, resulting in a net muscle protein accretion. Damas et al. [36] showed that following initial bouts of strength-training of the lower limbs, myofibrillar protein synthesis was highest when Z-line streaming, as an indicative of muscle damage, was most increased. After the initial period, the rates of myofibrillar protein synthesis were correlated to muscle hypertrophy. Therefore, it appears that the initial increases in muscle protein synthesis rate in the very early phase of strength-training are mainly “modified or repair-oriented” and not direct to hypertrophy [36,37]. Since the stimulation of muscle protein synthesis after EAA intake affects contractile myofibrillar protein as well as sarcoplasmic proteins [38], we hypothesized that EAA supplementation ingested immediately after and the days following eccentric exercise may have a therapeutic effect on muscle and connective tissue damage, reducing the overall release of markers of muscle damage by regulating muscle protein synthesis and modulating the remodelling of muscle and connective tissue.

Based on the CK response to eccentric exercise, Chen et al. [21] classified participants in 4 different categories ranging from low responders (less than 500 IU/L), medium responders (from 500 to 2000 IU/L), high responders (from 2000 to 10,000 IU/L) and higher responders (exceeding 10,000 IU/L). If we apply the same classification as Chen et al. [21] to our study, PLA group is composed of 3 low responders, 2 medium responders, 3 high responders and 3 higher responders, while EAA group is composed of 2 low responders, 4 medium responders, 2 high responders and 4 higher responders, indicating a similar distribution between lower and higher responders in both groups.

In our study, CK and Mb releases were significantly increased at D+3 and D+4, respectively at D+3 in PLA group only, but no statistical differences were observed between groups. As we observed a very large individual biological variability and because markers of muscle damage did not peak at the same time point for all subjects, we calculated a best-fit curve for CK and Mb release in order to have an overall view of the time course of both markers of muscle damage during one week of recovery. We found different curves between EAA and PLA groups for both markers. A smaller curve was also observed when looking at the high responders only. These findings indicate that a regular EAA

intake after and the days following an intensive eccentric exercise could have a positive effect on the overall time course of muscle damage markers release and that this positive effect may be more pronounced in high responders.

Because of their role as a regulator of the intracellular signalling pathways of muscle protein synthesis, the anabolic effect of nutrition is widely attributed to branched-chain amino acids (BCAA) (leucine, isoleucine and valine), and especially to leucine [39,40]. Nevertheless, Louard et al. [41] observed a reduced muscle protein turnover during BCAA infusion, as a result of a simultaneous reduction of muscle protein synthesis and muscle protein breakdown. Therefore, availability of all EAA seems to be a requisite to significant muscle protein synthesis stimulation [42]. In absence of EAA ingestion, the free EAA intracellular pool is the only source and since muscle protein breakdown is decreased during BCAA infusion, muscle protein synthesis is consequently reduced. Churchward-Venne et al. [43] showed that 5 g of leucine improves the efficiency of a low-protein beverage on muscle protein stimulation. The 2.85 g leucine content of our supplement may have been too small to induce maximum muscle protein synthesis and may have alleviate the impact of such a supplement on the recovery of exercise-induced muscle damage.

In our study, EAA intake immediately after and the days following an intensive eccentric exercise session did not seem to have any effect on muscle soreness as no significant differences were found between groups in the time course of recovery, respectively the peak value of the VAS score. Schwane et al. [44,45] used distinct modified scales adapted from DeVries [46] to rate the perceived muscular soreness and applied them to the various muscle groups that were prone to DOMS in his studies. Although the values of the scores differed depending on the study, they were classified as following: a first score corresponding to the value 0 represented complete absence of muscle soreness, a second score was defined by light pain felt only during palpation, a third score by moderate pain, some stiffness and/or weakness, especially during movement and the highest score by severe pain limiting the range of motion. The statistical analysis focused on the various muscle groups, but as muscle soreness affected muscle groups differently depending on the subjects, a peak rating and a mean score were also calculated. In a later study, Schwane et al. [47] dropped the modified scale adapted from DeVries [46] and chose to use a simplified subjective rating scale ranging from 0 (“no pain at all”) to 100 (“worst pain imaginable”) to assess muscle soreness. The modified, more cumbersome scale adapted from DeVries [46] may be appropriate to estimate the overall sensation of discomfort in daily life activities after moderate exercise, but since the exercise protocol we set up was a heavy and unaccustomed eccentric exercise of the upper limbs, we found it more adequate to use the simplified rating scale. We assumed that it would be more sensitive in subjects experiencing severe muscular soreness as the VAS score allowed a scaling of the intensity of the pain experienced by the subjects over the days. Furthermore, as the proportion of the subjects with impaired muscle function and displaying high VAS score was similar across the PLA and the EAA group, we don’t think that the rating scale used by Schwane et al. [44,45] in his early investigations would have elicited more differences between groups.

We cannot rule out that the use of a similar scale [44,45], probably more appropriate to estimate the overall sensation of discomfort of daily life activities, would have elicited differences between the PLA and the EAA group. However, subjects displaying high VAS score during palpation also had severe sensations of discomfort affecting movement and the proportion of those subjects was comparable among groups.

5. Conclusion

To conclude, this study showed that a heavy-load eccentric exercise of the upper limb could cause very large rises in plasma levels of the markers of muscle damage and that the high individual variability could be to some extent related to muscle effort undergone during the exercise. Moreover, EAA supplementation ingested during the week following the eccentric exercise could have minor effects on the overall plasmatic CK and Mb release during the recovery period, indicating a faster muscle remodelling with no impact on delayed muscle soreness. However, further research with larger samples is needed in order to classify the subjects according to their susceptibility to develop exercise-induced muscle damage and consequently reduce the variability of the sample.

Disclosure of interest

The authors declare that they have no competing interest.

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