Effect of soy supplementation on inflammation and lactic acid induced by exhaustive exercise in rats

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Summary

Introduction: Exhautive physical exercise generates inflammatory and lactic acid markers. The supplementation with natural substances is reason for analysis due to its limited side effects.
Objective: To determine the inflammatory response and the level of lactic acid induced by exhaustive physical exercise after the ingestion of soybean in animal model.
Materials and method: Thirty male Sprawley dawley rats from 180 to 200 g were used, healthy divided into three groups: sedentary (C), with soybean intake tested (E+TP) and without soybean intake tested (E). The E + TP and E groups performed the Morris Water Maze Test. Inflammatory markers were determined as tumor necrosis factor alpha (TNF-a), interleukin 1 beta (IL-1β), interleukin 6 (IL-6) in plasma by ELISA technique, enzyme cyclooxygenase 2 (COX-2), nitric oxide synthase (iNOS) and as anti-inflammatory marker Peroxisome proliferator-activated receptor gamma (PPAR-γ), which was measured in quadriceps muscles by Western-blot technique and measured lactic acid in blood.
Results: A significant decrease in plasma was obtained in the inflammatory levels of TNF-α (600 vs 350 pg/ml), IL-1β (450 vs 150 pg/ml), and IL-6 (480 vs 100 pg/ml), COX-2 (52 vs. 25 RDU) and iNOS (58 vs. 8 RDU) in the E+TP group compared to the E group. In addition an increase in the expression of the PPAR-γ protein was observed (18 vs 65 RDU) in the group E+TP compared to group E. Regarding the measurements of lactic acid, the groups obtained maximum values of: E: 35, C: 22 and E+TP: 28 Mmol/Lactate, which indicates that Group E and E+TP although they underwent the same test, lactic acid levels are heterogeneous.
Conclusion: The intake of soy mitigates the levels of lactic acid and inflammatory markers induced by exhautive physical exercise in animal models.

Key words:
Inflammation. Lactic acid. Soy. Exercise. Exhaustive.

Efecto de la suplementación con soja sobre la inflamación y ácido láctico inducido por ejercicio físico exhaustivo en ratas

Resumen

Introducción: El ejercicio físico exhaustivo genera marcadores inflamatorios y de ácido láctico. La suplementación con sustancias naturales es motivo de análisis debido a sus escasos efectos secundarios.
Objetivo: Determinar la respuesta inflamatoria y el nivel de ácido láctico inducidos por ejercicio físico exhaustivo después de la ingesta de soja en modelo animal.
Materiales y método: Se emplearon treinta ratas macho de raza Sprawley dawley de 180 a 200 g, sanos divididos en tres grupos: sedentario (C), con ingesta de soja a prueba (E+TP) y sin ingesta de soja a prueba (E). Los grupos E+TP y E, realizaron la prueba Morris Water Maze Test. Se determinaron marcadores inflamatorios como factor de necrosis tumoral alfa (TNF-a), interleuquina 1 beta (IL-1β), interleuquina 6 (IL-6) en plasma mediante técnica ELISA, enzima ciclooxigenasa 2 (COX-2), óxido nítrico sintaza (iNOS) y como marcador antiinflamatorio Receptor gamma activado por proliferador de peroxisoma (PPAR-γ), el cual, se midió en músculos cuádriceps mediante técnica de Western-blot y se midió el ácido láctico en sangre.
Resultados: Se obtuvo una disminución significativa en plasma de los niveles inflamatorios de TNF-α (600 vs 350 pg/ml), IL-1β (450 vs 150 pg/ml), e IL-6 (480 vs 100 pg/ml), COX-2 (52 vs 25 RDU) e iNOS (58 vs 8 RDU) en el grupo E+TP en comparación con el grupo E. Además se observó un aumento de la expresión de la proteína PPAR-γ (18 vs 65 RDU) en el grupo E+TP en comparación con el grupo E. Respecto a las mediciones de ácido láctico los grupos obtuvieron valores máximos de: E:35, C:22 y E+TP:28 Mmol/Lactato, lo cual, indica que el grupo E y E+TP a pesar que se sometieron a la misma prueba, los niveles de ácido láctico son heterogéneos.
Conclusión: La ingesta de soja mitiga los niveles de ácido láctico y de marcadores inflamatorios inducidos por el ejercicio fìsico exhautivo en modelo animal.

Palabras clave:
Inflamación. Ácido láctico. Soja. Ejercicio. Exhaustivo.

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Introduction

Scientific evidence links the beneficial effects of soy consumption with decreased oxidative stress after exercise. Similarly, soy supplementation combined with moderate exercise could have a beneficial effect on the lipid profile in ovariectomised rats. In this context, it is worth considering that exhaustive endurance exercise may be associated with physiological responses through markers which determine peripheral inflammatory activation as an immune response to protect and stabilise homeostasis[1,2]. In response to inflammation, circulating cytokines like TNF-α, IL-6, IL-10 and IL-1β increase following exercise, evidenced in high-intensity exercise chiefly in the form of muscle and organ damage, and suppression of the immune barrier[3-5].

To reduce the inflammatory damage caused, nonsteroidal anti-inflammatory drugs (NSAIDs), among the drugs the World Health Organization (WHO) promotes for their analgesic, anti-inflammatory and antiplatelet effects, have been used6. Given that exercise is the gradual summation of physical stimuli, the use of substances such as NSAIDs in endurance sports is evident[7], as seen from their use at the 2007 Pan American Games[8] and the Sydney 2000 Summer Olympics[9,10]. However, it should be noted that taking NSAIDs is related to a deterioration of the renal and digestive systems, and cardiovascular problems such as hypertension[11-13].

Meanwhile, natural substances are available which possess antioxidant and anti-inflammatory properties, such as the flavonoids found in fruits, vegetables and seeds, natural compounds known as nutraceuticals, phytonutrients or phytochemicals[14,15]. These include phenolic compounds, isothiocyanates, terpenoids, alkaloids, polyunsaturated fatty acids and flavonoids, metabolite subgroups with anti-inflammatory functions[16-20].

One of these substances is found in soy, which is rich in flavonoids and shows beneficial effects for the health through isoflavones and proteins that are potentially effective for human health and the prevention of various chronic diseases due to their anti-inflammatory effect[21,22].

Materials and methods

Sample

Experimental study conducted on thirty 12-month-old Sprawley Dawley rats belonging to the laboratory of the University of Valencia (Spain) with an average weight of 190 g, bred and kept in hygienic conditions with controlled temperature, humidity and light/dark cycle (12/12 hours), in compliance with regulations (Directive 86/609/EEC, OJ L358.1, 12 December 1987 and the National Institutes of Health's Guide for the Care and Use of Laboratory Animals, NIH. Publ. No. 85-23, 1985) and with the approval of the institution's ethical committee (AP-073/09).

Procedure

The necessary measures were taken to minimise the pain and discomfort of the laboratory animals. 3 groups were used, each consisting of n: 10, with the following intervention: control group (C), soy intake group and performance of the Morris Water Maze task[23] (E+TP) and group without soy intake with intervention (E). The three groups performed the Morris Water Maze task without prior training and with a proportional increase of 35% gravitational body weight, showing average lactate levels of 28 mmol/lactate, an indicator of exhaustive effort through quasi submersion in water[24]. The group with soy intake was given 0.5 g per kilogram of body weight. All the rats used in the experiment were euthanised immediately after the Morris Water Maze procedure.

Measurements

Inflammatory markers were measured: tumour necrosis factor alpha (TNF-a), interleukin 1β (IL-1β) and interleukin 6 (IL-6) in plasma using ELISA, and cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in soleus using the western blot technique. The anti-inflammatory marker peroxisome proliferator-activated receptor gamma (PPAR-γ) and lactic acid in plasma were also measured.

Statistical analysis

The values were expressed as mean±SD. Parametric normality tests were applied. Differences between groups were evaluated by one-way ANOVA for independent samples. Statistical significance was accepted at $p \leq 0.05$. The data sets in which F was significant were examined by a modified t- test.

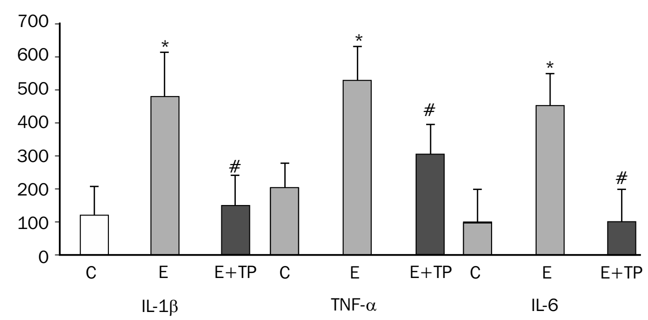
Results

The results showed a significant decrease in the E+TP group compared to the E group in inflammatory levels of TNF-α, IL-1β and IL-6 in plasma (Figure 1), and the expression of COX-2 and iNOS proteins (Figure 2), along with an increase in the expression of the PPAR-γ protein (Figure 3). The inflammation values of the E+TP group were similar to those of group C. Lactic acid levels decreased with soy intake (Figure 4).

Discussion

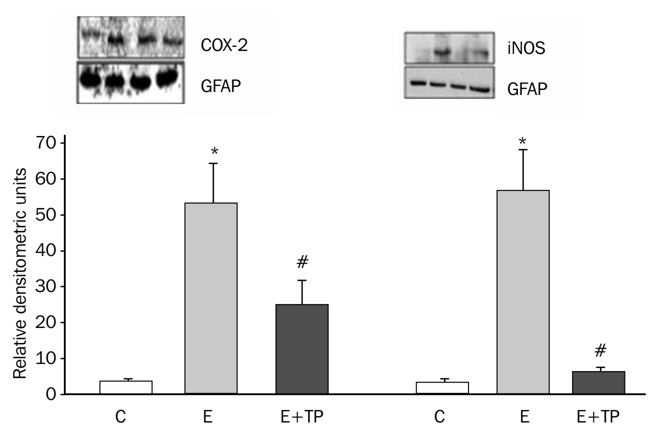
Muscle damage is evident due to the incidence of physical exercise. Different studies have identified an increase in creatine kinase (CK) in both older and younger individuals[25,26]. In our study, we observed higher levels of lactic acid when the Morris Water Maze procedure adapted with an increase in gravitational weight was performed. In this context, the oxidative balance of the human body is essential for metabolic regulation, the production of metabolic energy, the activation and inactivation of biomolecules, signal transduction, cell turnover and control of vascular tone, among other things. If this equilibrium between the oxidising systems (reactive species generators) and antioxidants is thrown off balance in favour of the former, by excessive production of

Figure 1. Levels of interleukin 1β (IL-1β), tumour necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) (pg/ml) in plasma.



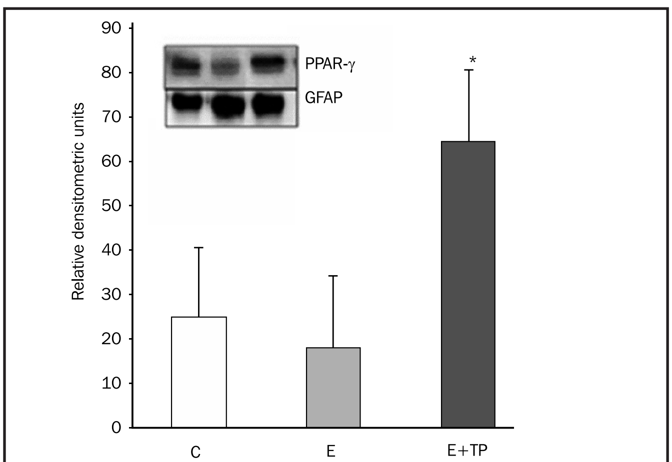
*p<0.05 vs control. #p<0.05 vs E+TP.

Figure 2. Protein expression of cyclooxygenase-2, COX-2 and inducible nitric oxide synthase (iNOS) (RDU) in quadriceps muscles.



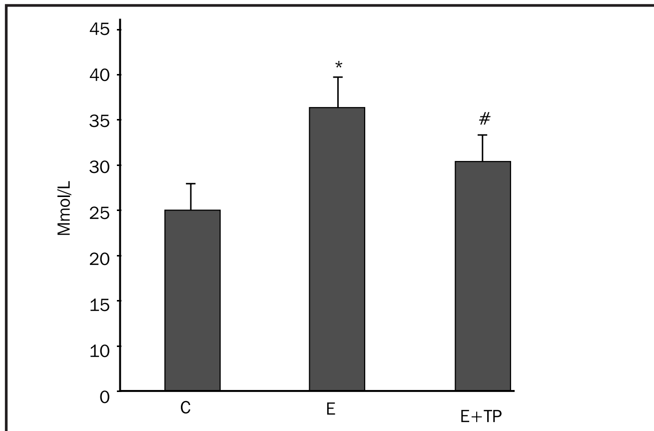
*p<0.05 vs control. #p<0.05 vs E+TP.

Figure 3. Levels of peroxisome proliferator-activated receptor gamma (PPAR-γ) (pg / ml) in plasma.



*p<0.05 vs control. #p<0.05 vs E+TP.

Figure 4. Lactic acid levels in plasma.



*p<0.05 vs control. #p<0.05 vs E+TP.

ROS and RNS, the weakening of the antioxidant systems is related to inflammation[27].

It is evident that free radicals play an important role as mediators of muscle damage and inflammation caused as a result of strenuous exercise. It is suggested that the generation of oxygen free radicals increases during exercise as a result of the increase in mitochondrial VO_2 and the greater flow of electrons in the transport chain due to a greater production of lactic acid and even the redistribution of blood flow that occurs in exertions of this kind[28].

Scientific evidence suggests that trained subjects show a greater overall antioxidant capacity than untrained subjects as a result of muscular endurance adapting to training, given that increased oxygen consumption would cause a greater increase in cell damage, which is offset by the higher antioxidant status of these subjects[29]. To help reduce this metabolic stress, some have proposed supplements to enhance the antioxidant defences of the system, but "those who consume more vegetables may naturally have an improved antioxidant defence system" without pills to counteract the oxidative stress induced by exercise, due to this increased amount[30]. This represents evidence consistent with the results of our study, which aimed to show the implication of soy for inflammatory levels through experimentation. It can be considered that plant foods are on average 64 times more antioxidant than meat and indeed contemplate a greater intake of foods which provide phytonutrients[31].

It is necessary to take into account fundamental mitigators such as the muscle damage caused by exercise, which has been widely documented. The sensation of pain and discomfort after moderate or exhaustive exercise is a product of muscle action and is compounded with greater eccentric actions, maximum intensity appearing between 24 and 48 hours after carrying out the physical activity[32]. From this perspective, our study establishes this biochemical condition, which is part of the preliminary hypothesis, in consideration of soy as an inhibitor of inflammatory processes shown in the sustained sample[33].

Vitamins C and E have been used to alleviate delayed onset muscular soreness (DOMS). Thompson *et al.* (2001) gave 16 men vitamins supplements (C and E) 14 days before causing muscle damage with intermittent running, showing that supplementation has beneficial effects, unlike Beaton *et al*. (2002), who gave 18 athletes the same vitamin supplements 30 days before the exercise. With their protocol, similar to the one used by Thompson *et al.,* Silva *et al.* (2010) concluded that supplementation with these vitamins plays an important role in the defence against DOMS and oxidative stress[34].

Connolly et al. (2006) concluded that drinking cherry juice before and after eccentric exercise reduced the symptoms of damage and aided recovery, which is relevant for comparison with the data thrown up by our study, a comparison which leads to consideration of a relationship between the two and ultimately identification of soy as an inhibitor of inflammatory processes[35,36].

It is concluded that exposure to the consumption of polyphenols promotes recovery from the muscle damage, inflammation and oxidative stress induced by exercise due to their immune response to inflammatory processes. The results of this study are consistent with those previously named, so justification by means of comparative studies is effective when it comes to accepting the prior hypothesis and a decrease in the expression of COX-2 and iNOS proteins ($p < 0.05$) is observed in group E+TP compared to group E. Group E+TP gives values similar to those of group C and shows an increase in the expression of the PPAR-γ protein ($p < 0.05$) compared to group E. E+TP gives values similar to those of group C.

Regarding the limitations of the study, we suggest that experiments be conducted with different tests to determine exhaustive exercise and that the supplementation be altered to include other substances in such a way as to question the effects on the inflammatory response.

Conflict of Interests

The authors declare that they are not subject to any type of conflict of interest.

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