Acute inflammatory responses to flexibility training: a systematic review

Respostas inflamatórias agudas ao treinamento de flexibilidade: uma revisão sistemática

Carlos José Nogueira1,2,3, Andrea Dos Santos Garcia3, Isabelle Vasconcellos de Souza1, Antônio Carlos Leal Cortez1,2,4, Gilmar Weber Senna1,3,5, Paula Paraguassu Brandão3, Estélio Henrique Martin Dantas1,3

1. Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Rio de Janeiro, RJ, Brazil
2. Força Aérea Brasileira (FAB), Escola Preparatória de Cadetes do Ar (EPCAR), Barbacena, MG, Brazil
3. Universidade Tiradentes (UNIT), Aracaju, SE, Brazil
4. Centro Universitário Santo Agostinho (UNIFSA), Teresina, PI, Brazil
5. Universidade Católica de Petrópolis, Petrópolis, RJ, Brazil

ABSTRACT
Objective: The aim of the study was to systematically assess the scientific evidence available on the effectiveness and safety of flexibility training at different intensities in terms of acute inflammatory responses in adult men. Methods: A search was conducted in the Medline/PubMed, Cochrane Library, Web of Science and Scopus databases and a manual search in the reference lists of relevant studies. The research question and strategy used were based on the PICO model. Included were studies involving adults aged between 18 and 45 years, published in English, Spanish and Portuguese, with no restriction for year of publication. Results: A total of 1014 articles were initially recovered. After duplicates were eliminated, 655 references were analyzed by title and abstract, 16 of which were included for reading in their entirety. After this stage, 13 references were excluded. At the end, three studies were considered eligible. Conclusion: The evidence available suggests that stretching exercises maximum in combination with non-habitual eccentric exercise or applied alone, are associated with a possible acute inflammatory response. Based on the evidence and the quality of the articles included in this review, the results should be interpreted with caution. Future research with better methodological quality involving the variables studied may better explain the results obtained to date.

Keywords: exercises; muscle stretching exercises; flexibility; inflammation.

RESUMO
Objetivo: O objetivo do estudo foi avaliar sistematicamente as evidências científicas disponíveis sobre a eficácia e segurança do treinamento de flexibilidade em diferentes intensidades sobre as respostas inflamatórias agudas em homens adultos. Métodos: Foi realizada uma busca nas bases de dados Medline/PubMed, Cochrane Library, Web of Science e Scopus e uma busca manual nas listas de referências de estudos relevantes. A questão de pesquisa e a estratégia utilizadas foram baseadas no modelo PICO. Incluídos estudos envolvendo adultos com idade entre 18 e 45 anos, publicados em inglês, espanhol e português, sem restrição de ano de publicação. Resultados: Um total de 1.014 artigos foram recuperados inicialmente. Após a eliminação das duplicatas, foram analisadas 655 referências por título e abstract, 16 de que foram incluídas para leitura em sua totalidade. Após esta etapa, 13 referências foram excluídas. Nos finais, três estudos foram considerados elegíveis. Conclusão: As evidências disponíveis sugerem que exercícios de alongamento máximo em combinação com exercícios excêntricos não habituais ou aplicados isoladamente estão associados a uma possível resposta inflamatória aguda. Com base nas evidências e na qualidade dos artigos incluídos nesta revisão, os resultados devem ser interpretados com cautela. Pesquisas futuras com melhor qualidade metodológica envolvendo as variáveis estudadas podem explicar melhor os resultados obtidos até o momento.

Palavras-chave: exercícios; exercícios de alongamento muscular; flexibilidade; inflamação.
Introduction

Flexibility training is considered a form of physical activity used by athletes, patients in rehabilitation and individuals engaged in physical activities [1]. Control of flexible training intensities enables differentiating between submaximal (stretching) and maximal (flexibilizing) exercises, which is essential to good physical planning and preparation [2,3].

Movement in submaximal stretching occurs within the normal joint range, slightly sustained, without pain or discomfort; in stretching exercises maximum, the muscle is stretched to the point of discomfort, a little before the pain threshold [4,5].

According to Behm et al. [6], four main stretching exercises techniques can be applied: static, dynamic, ballistic, and proprioceptive neuromuscular facilitation (PNF). The static technique involves a continuous controlled movement for the range of final motion of a single or multiple joints actively contracting the agonist muscles (active static) or using external forces such as gravity, a partner or stretching aids (passive static). In the final position, the individual maintains the muscle in a stretched position for a certain time [6].

The magnitude of the moment of force (or torque) that will be applied to a joint or set of joints during flexibility training is characterized as intensity, while the number of sets and time are the dimensions of volume [7]. Special importance should be attributed to training intensity, since a small moment of force may result in a viscoelastic response of the locomotor apparatus with little or no gain in range of motion. However, applying excessive force may compromise the tissue, resulting in inflammation or even injury [8,7].

An experiment with adult male mice reported high neutrophil levels after passive stretching exercises maximum protocol, exhibiting an acute inflammatory response, given that activated neutrophils secrete proinflammatory cytokines such as interleukin 1 beta (IL-1β), tumour necrosis factor alpha (TNF-α) and Interleukin 6 (IL-6) [9]. This inflammatory response to training plays an essential role in energy metabolism, skeletal muscle repair and remodeling, and anabolic/catabolic response, and may respond differently according to exercise type, intensity, volume and recovery between the exercise phases [10].

Thus, different substrates of the inflammatory response are produced and secreted, such as cytokines, which belong to a group of regulatory glycoproteins produced by leukocytes and tissues such as the skeletal muscles [11] and are responsible for the interconnections between immunological cells as a response to infection or tissue damage, with possible proinflammatory and anti-inflammatory activity [12,13].

Evidence on the inflammatory responses caused by flexibility training are scarce in the literature. This hampers a better understanding of the physiological mechanisms of adaptation resulting from alterations in this methodological training variable and the monitoring of adaptive responses to establish a balance between the overload applied and recovery [14].
Thus, investigating the inflammation resulting from flexibility training may complement the information presented by traditional tissue injury markers already evident in the literature, such as creatine kinase (CK) or delayed-onset muscle soreness (DOMS) [15].

Therefore, the aim of the present review was to systematically assess and summarize the scientific evidence available regarding the effectiveness and safety of flexibility training at different intensities (maximal and submaximal) on acute inflammatory responses in adult men.

**Methods**

The systematic search in the literature was conducted in line with the guidelines of the PRISMA Statement reports for systematic reviews and meta-analysis [16] and the Cochrane Handbook for Systematic Reviews of Interventions [17]. The current review was registered at the International Prospective Register of Systematic Reviews (PROSPERO), under protocol number 42020165515, and entitled “Acute inflammatory responses to stretching”.

**Search strategy**

A search was conducted in the following electronic databases: Medical Literature Analysis and Retrieval System Online (Medline, via PubMed), Cochrane Library, Web of Science and Scopus. The search strategies created and used for the databases are presented in Table I. A manual search was carried out in the reference lists of the relevant studies to identify eligible articles not found in the electronic search. The searches were performed in June 2020 and update in July 2021.

The following descriptors were selected in the Descriptores em Ciências da Saúde (DeCS) and Medical Subject Headings (MeSH) databases: *homens* (men), *adulto* (adult), *exercícios de alongamento muscular* (muscle stretching exercises), *amplitude de movimento articular* (joint range of motion), *inflamação* (inflammation), *citocinas* (cytokines), *biomarcadores* (biomarkers), as described and presented along with the search strategy used in Medline via Pubmed and adapted to other databases (Chart 1).
<table>
<thead>
<tr>
<th>Database</th>
<th>Search Strategy</th>
<th>Results</th>
</tr>
</thead>
</table>
| **Medline** (PubMed) | #1 “Men”[Mesh] OR (Boys)  
#2 “Adult”[Mesh] OR (Adults)  
#3 #1 OR #2  
#4 “Muscle Stretching Exercises”[Mesh] OR (Exercise, Muscle Stretching) OR (Exercises, Muscle Stretching) OR (Muscle Stretching Exercise) OR (Static Stretching) OR (Stretching, Static) OR (Passive Stretching) OR (Stretching, Passive) OR (Relaxed Stretching) OR (Stretching, Relaxed) OR (Static-Passive Stretching) OR (Static Passive Stretching) OR (Stretching, Static-Passive) OR (Isometric Stretching) OR (Stretching, Isometric) OR (Active Stretching) OR (Stretching, Active) OR (Static-Active Stretching) OR (Static Active Stretching) OR (Stretching, Static-Active) OR (Ballistic Stretching) OR (Stretching, Ballistic) OR (Dynamic Stretching) OR (Stretching, Dynamic) OR (Proprioceptive Neuromuscular Facilitation Stretching)  
#5 “Range of Motion, Articular”[Mesh] OR (Joint Range of Motion) OR (Joint Flexibility) OR (Flexibility, Joint) OR (Range of Motion) OR (Passive Range of Motion)  
#6 #4 OR #5  
#7 “Inflammation”[Mesh] OR (Inflammations) OR (Innate Inflammatory Response) OR (Inflammatory Response, Innate) OR (Innate Inflammatory Responses)  
#8 “Cytokines”[Mesh]  
#9 “Biomarkers”[Mesh] OR (Markers, Biological) OR (Biologic Markers) OR (Markers, Biologic) OR (Biologic Marker) OR (Marker, Biologic) OR (Marker, Biological) OR (Biological Marker) OR (Biological Markers) OR (Markers, Laboratory) OR (Laboratory Markers) OR (Laboratory Marker) OR (Marker, Laboratory) OR (Serum Markers) OR (Markers, Serum) OR (Marker, Serum) OR (Serum Marker) OR (Surrogate Endpoints) OR (Endpoints, Surrogate) OR (Surrogate End Points) OR (End Points, Surrogate) OR (Surrogate End Point) OR (End Point, Surrogate) OR (Surrogate Endpoint) OR (Endpoint, Surrogate) OR (Markers, Clinical) OR (Clinical Markers) OR (Clinical Marker) OR (Marker, Clinical) OR (Viral Markers) OR (Markers, Viral) OR (Viral Marker) OR (Marker, Viral) OR (Biochemical Markers) OR (Markers, Biochemical) OR (Marker, Biochemical) OR (Biochemical) OR (Markers, Immunologic) OR (Immunologic Markers) OR (Immunologic Marker) OR (Marker, Immunologic) OR (Immunologic Markers) OR (Immune Markers) OR (Marker, Immune) OR (Immunologic Marker) OR (Immune Markers) OR (Surrogate Markers) OR (Markers, Surrogate) OR (Marker, Surrogate) OR (Surrogate Marker)  
#10 #7 OR #8 OR #9  
#11 #3 AND #6 AND #10  
Filtros aplicados: Adult (19-44 years), Male | 108 |
| **Cochrane Library** | #3 #1 OR #2  
#6 #4 OR #5  
#10 #7 OR #8 OR #9  
#11 #3 AND #6 AND #10  
Filtros aplicados: Trials | 365 |
| **Web of Science** | #3 #1 OR #2  
#6 #4 OR #5  
#10 #7 OR #8 OR #9  
#11 #3 AND #6 AND #10  
Filtros aplicados: Article | 210 |
| **Scopus** | TITLE-ABS-KEY(“(Men) OR (Adult) AND (Muscle Stretching Exercises) OR (Range of Motion, Articular) AND (Inflammation) OR (Cytokines) OR (Biomarkers)) AND (LIMIT-TO (DOCTYPE,”ar” ))  
Filtros aplicados: Article | 329 |
| **Total** | ------- | 1012 |
Research question

The research question and strategy used in this study were based on the Population, Intervention, Comparison, Outcome (PICO) model, commonly applied in evidence-based practice and recommended for systematic reviews [18].

Thus, adult men, trained or not in flexibility, were used as the “Population”; for Intervention, studies involving different intensities of flexibility training were considered, for Control, the “not applicable” criterion was adopted; and “Outcomes” were the primary and secondary outcomes of acute inflammatory responses caused by flexibility training exercises. Thus, the final PICO question was “Does flexibility training at different intensities increases the acute inflammatory response in adult men?”

Eligibility criteria

Included were studies involving adults aged between 18 and 45 years and complete articles published in English, Spanish and Portuguese. There was no restriction for year of publication.

With respect to study designs, given the limited number of studies published to date and that the aim of this review was to map knowledge, randomized and non-randomized (quasi-experimental) clinical studies were included.

The following exclusion criteria were established: studies other than randomized, quasi-randomized or experimental clinical trials, studies involving older adults, children, individuals with disabilities, limitations, or chronic diseases; studies with high-performance athletes and those using animal models.

Study selection

The study selection process was performed by two independent reviewers, with disagreement resolved by a third reviewer. The articles were selected in two stages. In the first stage, titles and abstracts of the references identified in the search were assessed and the potentially eligible studies were pre-selected. In the second stage, the entire text of the pre-selected studies was evaluated to confirm eligibility. The selection process was conducted using the Rayyan platform (https://rayyan.qcri.org) [19]. The entire inclusion and exclusion process was in accordance with the PRISMA FLOW stages, illustrated in Figure 1.

Studies included

After the selection process, the following studies were included: one randomized clinical trial [20] and two quasi-experimental non-randomized studies [8,21].

Data extraction

Standardized electronic forms were used for this stage. The reviewers independently extracted data related to the morphological characteristics of the studies, interventions and results. The differences were resolved by consensus. The following
data were initially collected: authors, year of publication, type of study, sample (number of participants), methods, intervention protocol and control group (if applicable), outcomes assessed, results and conclusions.

**Assessment of the methodological quality of the studies included**

The methodological quality and/or risk of bias of the studies were independently assessed by two reviewers using the appropriate tools for each study design, as follows: randomized clinical trial - Cochrane risk of bias [22], non-randomized or quasi-experimental clinical trials - ROBINS-I [23]. The assessment of risk of bias of randomized clinical trials is summarized in Figure 2 and of non-randomized or quasi-experimental trials in Chart II.

**Results**

**Search results**

The search produced 1014 studies. After duplicates were eliminated, 655 references were analyzed by title and abstract, resulting in 16 inclusions (according to the PICO question) for reading in their entirety. After this stage, 13 references were excluded (different populations, interventions and/or outcomes). At the end, three studies were considered eligible and analyzed. The flowchart of the study selection process is presented in Figure 1, and Table I summarizes the characteristics of these studies.

**Study results**

Since only one randomized clinical trial was eligible for review [20], we were unable to conduct a quantitative summary between the studies. Thus, a narrative approach was more appropriate.

The qualitative summary obtained for the acute inflammatory responses are presented in Table I

**Risk of bias assessment**

In general, considering the Cochrane tool, the randomized clinical trial showed high risk of bias and unclear risk in three domains, and low risk of bias only in the random sequence generation domain, as shown in Figure 2. The quasi-experimental studies according to the ROBINS-I tool exhibited a serious and critical risk of bias in most of the domains assessed, and only one study showed low risk of bias in the incomplete outcome data domain since data were missing, as illustrated in Chart II.
Adapted from Page et al. [16]

Figure 1 - Flowchart of the study selection process (PRISMA Flow)

Figure 2 – Risk of bias of the randomized clinical trial Apostolopoulos et al. 2018 [20], using the Cochrane Risk of Bias table
### Table I – Characteristics of the studies included

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Participants</th>
<th>Intervention/control protocol</th>
<th>Duration</th>
<th>Outcome</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apostolopoulos et al., 2018 [20]</td>
<td>RCT</td>
<td>30 recreationally active men (age: 25 ± 6 years)</td>
<td>Submaximal Stretching (30 to 40% of perceived ROM) performed after the non-habitual eccentric protocol (n= 10). Stretching exercises maximum (70 to 80% of perceived maximum stretching) performed after the non-habitual eccentric exercise protocol (n = 10). Non-habitual eccentric exercises without stretching (control) (n =10).</td>
<td>3 x 60 s per muscle group.</td>
<td>Assessment of pre-exercise hsCRP level, and 24, 48 and 72 hours post.</td>
<td>Stretching exercise submaximal and maximum: small harmful effect of low-intensity passive static stretching on hsCRP from baseline to 24 h after eccentric exercise, when compared with high-intensity passive static stretching. Submaximal stretching x Control: was unclear for all comparisons of the points in time evaluated. Stretching exercises maximum x Control: small harmful effect on hsCRP from baseline to 72 h after non-habitual eccentric exercise.</td>
</tr>
<tr>
<td>Apostolopoulos et al., 2015 [21]</td>
<td>ECQR</td>
<td>11 recreational athletes (age: 26 ± 6.2 years)</td>
<td>Flexibility training of right ischiotibial muscle to 30% of maximal ROM. Flexibility training of right ischiotibial muscle to 60% of maximal ROM. Flexibility training of right ischiotibial muscle to 90% of maximal ROM.</td>
<td>5 x 60 s with 10s rest between sets.</td>
<td>Assessment of pre-exercise hsCRP levels and 24 h post, for both conditions.</td>
<td>Significant increases in hsCRP levels were observed between 30 and 90% (p = 0.004) and 60 and 90% (p = 0.034), but not between 30 and 60% (p&gt; 0.05).</td>
</tr>
<tr>
<td>Apostolopoulos et al., 2015 [8]</td>
<td>QRCT</td>
<td>12 recreationally active men (age: 29 ± 4.33 years)</td>
<td>Ischiotibial, gluteal and quadriceps stretching exercises. Similarly to the stretching exercises maximum intervention, participants rested on a rug in the supine position, with knees supported for 10 minutes. This position was held for more than 18 minutes, imitating the time allocated for the stretching exercises maximum intervention.</td>
<td>3 x 60 s until the point of discomfort or mild pain (stretching exercises maximum)</td>
<td>Assessment of pre-exercise hsCRP, IL-6, IL-1β and TNF-α levels and 24 h post for both conditions.</td>
<td>hsCRP increased significantly at 24h compared to controls and immediate post-stretching exercises maximum, for time (p = 0.005) and time x condition (p = 0.006). No significance was observed for IL-6, IL-1β or TNF-α.</td>
</tr>
</tbody>
</table>

RCT = Randomized clinical trial; QRCT = Quasi-randomized clinical trial; hsCRP = high-sensitivity C-reactive protein; ROM: Maximal range of motion; IL-6 = Interleukin 6; IL-1β = Interleukin 1 beta; TNF-α = Tumor necrosis factor alfa
Chart II – Summary of the risk of bias of non-randomized comparative studies, using the ROBINS-I tool

<table>
<thead>
<tr>
<th></th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
<th>D6</th>
<th>D7</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apostolopoulos et al., 2015 [21]</td>
<td>X</td>
<td>!</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>X</td>
<td>!</td>
<td>!</td>
</tr>
<tr>
<td>Apostolopoulos et al., 2015 [8]</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>!</td>
<td>X</td>
<td>!</td>
<td>!</td>
</tr>
</tbody>
</table>

Green (+) = low risk of bias; Yellow (-) = moderate risk of bias; Red (X) = serious risk of bias; Wine-colored (!) = critical risk of bias.

Domains:
- D1: Bias related to confounding factors;
- D2: Bias related to participant selection;
- D3: Bias related to intervention classification;
- D4: Bias related to deviations from intended interventions;
- D5: Bias related to missing data;
- D6: Bias related to outcome assessment;
- D7: Selection bias in the report of results.

Assessment certainty of evidence
We were unable to assess certainty of evidence for the outcome of interest of the present review, given that only one study (randomized clinical trial) evaluated the effect magnitude.

Discussion

The evidence of three clinical trials with available data whose primary and secondary outcomes were the inflammatory effects caused by flexibility training at different intensities: one randomized clinical trial [20] and two quasi-experimental clinical trials [8,21].

Considering the results of the randomized clinical trial for the outcome analyzed [20], there was a small harmful effect of submaximal stretching at the hs-CRP concentrations assessed from baseline to 24 hours after eccentric exercise when compared to stretching exercises maximum. The effects of submaximal stretching compared to controls were unclear for all the comparisons at the assessment times of hsCRP levels. However, there was a small harmful effect of stretching exercises maximum compared to controls on hsCRP from baseline to 72h after non-habitual eccentric exercise.

According to the results of this randomized clinical trial [20], stretching exercises maximum may have caused a slight inflammation, demonstrated by the increase in hsCRP concentrations after non-habitual eccentric exercise, with statistically higher hsCRP values at 24 h versus 72 h (p = 0.012).

In one of the quasi-experimental clinical trials, static flexibility training at different maximal ROM (30, 60 and 90%) in the right ischiotibial muscle promoted significant increases in hsCRP levels between 30 and 90% (p = 0.004) and 60 and 90% (p = 0.034), but not between 30 and 60% (p > 0.05), revealing that increases in the...
percentage of maximal ROM (intensity) are associated with a rise in hsCRP levels, causing possible systemic inflammation [21].

In the second quasi-experimental study, Apostolopoulos et al. [8] observed that stretching exercises maximum with three 60-second sets of static insistence to the point of discomfort or mild pain caused an acute inflammatory response sustained by the significant increase in hsCRP at 24h compared to the control condition and immediate post-stretching, for time (p = 0.005) and time x condition (p = 0.006). However, no significant increases were observed for inflammatory markers IL-6, IL-1β or TNF-α.

Comparing the results of the studies included in this review showed that passive stretching exercises maximum is associated with a likely increase in acute inflammatory response. Thus, stretching exercises maximum promoted higher concentrations of hsCRP, as clearly demonstrated in the quasi-experimental studies, in which flexibility training was applied alone, without resistance exercise.

The force generated by the acute stretch (mechanical stimulus) causes an excessive overload of the contractile elements of the skeletal muscle, exceeding their usual demands and inducing tissue damage [24]. Structurally, there is a myofilament disarrangement in the sarcomeres, damage to the sarcolemma, loss of fiber integrity and the subsequent leakage of muscle proteins into the blood [25]. This functional change causes a reduction or loss of muscle strength and is responsible for triggering an acute response [24].

The application of this overload causes microtraumas of varying degrees in the striated skeletal muscle tissue, connective tissue and bone tissue. These microtraumas, considered as temporary and repairable damage, result in an acute inflammatory response, instrumented by numerous specific chemical mediators such as C-reactive protein (CRP) and the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α, derivatives of the injured tissues [13,26-29]. The extent of the inflammatory response is determined by the degree of muscle damage, the magnitude of inflammation, and the lesion-specific interaction between the invading inflammatory cells and the injured muscle [24,28].

Considering intensity as an important parameter of flexibility training [26], evidence indicates that the magnitude of force applied to the muscle during stretching is a catalyst for tissue damage and inflammation [28,30,31] as observed in animals studies [9,32] and according to the results presented in this review.

In regard to the applicability and quality of the evidence, the studies included in the present review revealed high and critical risk of bias in assessment of the randomized clinical and quasi-experimental trials [8,20,21], respectively.

However, the findings of the present review need to be interpreted considering the following limitations: few studies were eligible, with only one randomized controlled trial and two quasi-experimental studies; the different study designs, experimental protocols and controls, measures of results and incomplete data in some of the studies hampered an additional quantitative synthesis; the conclusions were based on relatively low-quality data and consequent high risk of bias; and important
methodological questions, such as the lack of allocation concealment, group comparison at baseline of the participants and assessor blinding, limited the strength of the study conclusions.

**Conclusion**

The evidence available in randomized and non-randomized trials suggests that stretching exercises maximum, in combination with non-habitual eccentric exercise, or applied alone, is associated with an acute inflammatory response. However, the estimates of these results are very low, which precludes definitive conclusions. The limitations inherent to the design and methodological quality (high or critical risk of bias) of the studies significantly reduced the reliability of all the results presented. Thus, new studies with better methodological quality, involving the variables studied, may better elucidate the results obtained to date.

**Potential conflict of interest**
No potential conflicts of interest relevant to this article have been reported.

**Financing source**
There were no external funding sources for this study.

**Authors' contributions**

**Conception and design of the research:** Nogueira CJ, Senna GW, Dantas EHM;

**Data collection:** Nogueira CJ, Brandão PP, Souza IV, Garcia, AS;

**Analysis and data interpretation:** Nogueira CJ, Cortez ACL, Brandão PP, Souza IV, Garcia, AS;

**Statistical analysis:** Not applicable;

**Obtaining financing:** Not applicable;

**Writing of the manuscript:** Nogueira CJ, Dantas EHM;

**Critical review of the manuscript:** Senna GW, Cortez ACL;

**Final revision of the manuscript:** Nogueira CJ, Brandão PP, Dantas EHM.

**Academic link**
This study is linked to the thesis of doctoral student Nogueira CJ, from the Stricto Sensu Post-Graduation Program in Nursing and Bioscience, Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Rio de Janeiro, Brazil.

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