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Reply to the Letter to the Editor on "Effects of Light-Emitting Diode Therapy on Muscle Hypertrophy, Gene Expression, Performance, Damage, and Delayed-Onset Muscle Soreness: Case-Control Study With a Pair of Identical Twins"

Cleber Ferraresi, PT, PhD,

Laboratory of Electrothermophototherapy, Department of Physical Therapy, Federal University of São Carlos, São Carlos, São Paulo, Brazil, Post-Graduation Program in Biotechnology, Federal University of São Carlos, São Carlos, São Paulo, Brazil, Optics Group, Physics Institute of São Carlos, University of São Paulo, São Carlos, São Paulo, Brazil, Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, Massachusetts and Department of Physical Therapy, Universidade do Sagrado Coração - USC, Bauru, São Paulo, Brazil

Danilo Bertucci, MSc,

Department of Physiological Sciences, Federal University of São Carlos, São Carlos, São Paulo, Brazil

Josiane Schiavinato, PhD,

Faculty of Medicine, University of São Paulo, Ribeirão Preto, São Paulo, Brazil and Center for Cell Therapy and Regional Blood Center of Ribeirão Preto São, Paulo, Brazil

Rodrigo Reiff, PhD,

Department of Medicine, Federal University of São Carlos, São Carlos, São Paulo, Brazil

Amélia Araújo,

Faculty of Medicine, University of São Paulo, Ribeirão Preto, São Paulo, Brazil and Center for Cell Therapy and Regional Blood Center of Ribeirão Preto, São Paulo, Brazil

Rodrigo Panepucci, MSc, PhD,

Faculty of Medicine, University of São Paulo, Ribeirão Preto, São Paulo, Brazil and Center for Cell Therapy and Regional Blood Center of Ribeirão Preto, São Paulo, Brazil

Euclides Matheucci, PhD,

Post-Graduation Program in Biotechnology, Federal University of São Carlos, São Carlos, São Paulo, Brazil

Anderson F. Cunha, PhD,

Department of Genetic and Evolution, Federal University of São Carlos, São Carlos, São Paulo, Brazil

Vivian Maria Arakelian, PT, PhD,

Post-Graduation Program in Bioengineering, University of São Paulo, São Carlos, São Paulo, Brazil

Michael R. Hamblin, PhD,

Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, Massachusetts, Department of Dermatology, Harvard Medical School, Boston, Massachusetts and Harvard-MIT Division of Health Sciences and Technology, Cambridge, Massachusetts

Nivaldo Parizotto, PT, PhD, and
Laboratory of Electrothermophototherapy, Department of Physical Therapy, Federal University of São Carlos, São Carlos, São Paulo, Brazil and Post-Graduation Program in Biotechnology, Federal University of São Carlos, São Carlos, São Paulo, Brazil

Vanderlei Bagnato, PhD
Optics Group, Physics Institute of Sao Carlos, University of São Paulo, São Carlos, São Paulo, Brazil

To the Editor

Several interesting concerns about methods and results were raised in the Letter to the Editor regarding the study by Ferraresi et al.,[1] which also suggest possible bias in the discussion. Although at first view, all concerns seem to be pertinent, most of them were based on different studies compared with the study by Ferraresi et al.[1] Therefore, these concerns require a detailed response to avoid a biased interpretation.

METHODOLOGICAL ISSUES

Ferraresi et al.[1] reported “Two 19-year-old male identical (monozygotic) twins, 1.72 m, 70 kg, college soccer players, living together and having the same habits and diet…,” contradicting the statement of the authors “… only characterized as college soccer players….” Both twins being characterized as college soccer players implies (1) their regular practice of physical activity and (2) their level of physical conditioning, which was higher than moderate, and higher or equal to the high classification reported in the International Physical Activity Questionnaire (www.ipaq.ki.se).

As mentioned in the letter, differences in muscle performance may occur in soccer athletes,[2,3] mainly in running and jumping,[2] even though these differences may be actually small (1%–3.8%).[2] On the other hand, maximal aerobic power (VO_2 max) is not a “… clearly distinguishing variable separating players of different standards.”[3] However, it is important to highlight that (1) Ferraresi et al.[1] did not predict or generalize that all athletes undertaking different types of training or exercise would have exactly the same magnitude as the results found[1] and (2) the use of the term *useful* does not necessarily mean that all the different applications would see the same size of benefit.[1] In addition, the study[1] enrolled monozygotic twins having the same habits, level of activity, and diet, and used blinded evaluators and placebo therapy all designed to exclude or minimize genetic disparities[4] and methodological bias. Finally, the discussion session[1] highlighted the need for future studies enrolling a greater number of volunteers.

Time within the season and playing experience of the twins were not explicitly clarified.[1] The study[1] was conducted during the preseason; the twins played in the same team, had the

same playing experience (at least 2 yrs), and performed the same regular physical exercise program three times a week during the last full season.

Concerns about familiarization sessions with one-repetition maximum (1-RM) test were based on a study that enrolled subjects with athletic background (rugby, soccer, hockey) in the detraining period (6 mos without any strength training).[5] This information should be emphasized in the letter, because detraining can affect neural drive, muscle coordination, disinhibition of Golgi tendon organs, and performance in maximal tests, leading to possible overestimation of maximal load in the range of 6%–15% for 1-RM session tests for leg extension and leg press, respectively.[5] However, this was not the case as reported in the study by Ferraresi et al.[1] Regarding intrarater and interrater reliability, the squat on one-leg 1-RM test[6] cannot be compared with the leg press exercise performed in the study by Ferraresi et al.[1] Moreover, this reference[6] reported clinically acceptable intrarater (intraclass correlation coefficient, 0.90) and interrater (intraclass correlation coefficient, 0.96) reliability for leg extension on the one-leg 1-RM test, exercise performed in the study by Ferraresi et al.[1] The reference[7] cited by the authors is not related to the 1-RM test. Finally, our research group has worked with strength exercises and maximal tests for years,[8–12] and in a recent study, a single session of testing for 1-RM was found to be reliable when subjects had practice in resistance/strength training for at least 3 mos.[12] Thus, we believe that the concerns and suppositions about possible overestimation have not been substantiated.

As the authors may know, the leg press is a closed kinetic chain exercise that involves cocontraction of knee extensor and knee flexor muscles.[13–15] Although hamstrings are not agonist muscles in the leg press exercise, they display electromyographic activity during knee flexion and knee extension in this exercise.[13–15] For these reasons, we decided to include hamstring muscles in the thigh muscle volume analysis.

Overestimation of the muscle volume in the study by Ferraresi et al.[1] cannot be justified with a reference reporting results for a squat exercise.[16] Squat and leg press are different exercises. There is higher electromyographic activity for quadriceps and hamstring muscles in squat than in leg press.[13–15] As the authors may know, the type of exercise, intensity, volume, and frequency affect the production of more or less muscle hypertrophy, or increased muscle volume.[17] Thus, comparisons between different exercises[1,16] are not appropriate.

The review study[17] reports only an average increase in quadriceps femoris muscle cross-sectional area of 8.5% with strength exercises, and a range of 1.1%–17.3%. Thus, the 5% increase in muscle volume seen with light-emitting diode therapy (LEDT) placebo, including quadriceps and hamstring muscles, is completely within the range reported,[17] contradicting the suggestion of underestimation.

Specifically, a 20% increase in muscle volume with real LEDT includes cross-sectional area of the hamstring muscles, because they are recruited in the leg press exercise.[13–15] Moreover, the most important finding was probably the difference in final muscle volume between both therapies (placebo and real LEDT) when combined with a strength-training program.

As stated in the study,[1] the hypertrophy assessment was blinded, that is, the evaluator did not know which of the twins received real or placebo LEDT. Therefore, any suggestion of bias in overestimation of real LEDT or underestimation for LEDT placebo is not reasonable. Ferraresi et al.[1] used already published methods[18,19] that report errors of 0.78% (300 images) in calculations of cross-sectional area, and less than 2% (41 images) in calculations of muscle volume (intrarater reliability).[18,19] We agree that it is important to establish intrarater and interrater reliability for muscle hypertrophy assessment, as well as for all assessments performed in the study. Otherwise, the need for intrarater and interrater reliability of only one assessment could be tendentious. Finally, the study[1] did not intend to establish intrarater or interrater reliability for hypertrophy or muscle volume assessments, or for that matter for the other assessments performed in the study. Otherwise, the study would have different objectives and methods.

ISSUES IN THE DISCUSSION

The term *inflammation*, reported by Ferraresi et al.,[1] refers to the expression of interleukin (IL)-1β (gene analyzed). Inflammatory response and muscle hypertrophy in the letter was based on studies that did not directly address the role of IL-1β in muscle damage and regeneration[20–22] and was also based on a study looking at the role of other interleukins (IL-6, IL-7, IL-8, IL-10, IL-13, and IL-15).[23] As the authors may know, IL-1β is a known marker of muscle atrophy signaling,[24] promoting myoblast proliferation, but associated with delays in differentiation.[25,26] Furthermore, real LEDT promoted down-regulation of myostatin gene expression, a known gene related to muscle atrophy signaling and inhibition of satellite cell activation.[27,28] Finally, the visual analog scale was consistently scored lower with real LEDT treatment. Therefore, we cannot agree with the suggestion of the authors to revise the sentence “Moreover, there would be the added benefit of reduction in muscle damage and atrophy and less inflammation and pain….”

The authors stated that “… inflammatory response and muscle damage are known to dramatically reduce after a few weeks of strength training….”[29] The study cited[29] had a lower weekly training frequency (two times a week) compared with the study of Ferraresi et al.[1] (three times a week). Thus, whereas the study[29] allowed around 72 hrs for muscle recovery between 2 consecutive training sessions in the same week, the study by Ferraresi et al.[1] allowed around 48 hrs. Moreover, the training intensity of the study[29] was based on maximum repetitions and not percentages of 1-RM as in the study by Ferraresi et al.,[1] producing variations in the number of repetitions (9–12 repetitions).[29] Although the study[29] had on average 120 repetitions a week and 10 wks of training program, the study by Ferraresi et al.[1] had on average 210 repetitions a week and 12 wks of training program. As reported in the study,[29] the levels of myoglobin and IL-6 increased significantly after the third week (T2) of the training program, contradicting the statement of the authors “… inflammatory response and muscle damage are known to dramatically reduce after a few weeks of strength training [14] and Ferraresi et al. [1] displayed similar results (decreasing creatine kinase (CK) and muscle soreness).” It is important to make clear that Ferraresi et al.[1] did not report the same results regarding CK and muscle soreness. In Figure 6 of the study by Ferraresi et al.,[1] it is clear that CK increased at the 13th training session, whereas muscle soreness measured by visual analog scale kept decreasing during the 25th and 36th

training sessions. Finally, the study cited[29] used different markers for muscle damage (myoglobin) and inflammation (IL-6), as well as did not use real-time polymerase chain reaction for IL-1β gene expression. Taking into account the lack of similarity between both studies,[1,29] comparisons as made by the authors are inadequate and inconclusive.

Indeed, it is impossible to be sure if there was, or there was not, any influence of the last training session on the gene expression analysis.[1] However, because the training program had occurred over 12 wks,[1] with training sessions three times a week,[1] it is much more likely that the entire training period was predominant on the effects on muscle adaptation and gene expression, rather than only the final training session. Such adaptation can be seen in the results for CK and delayed muscle soreness that were observed throughout the whole study.

The statement made by Ferraresi et al.,[1] "On the other hand, patients suffering from inflammation, pain, loss of muscle mass and strength, and muscle atrophy as result of orthopedic surgical procedures…," can indeed be understood as containing some extrapolation. However, the idea behind that sentence was to suggest the possible clinical practice of LEDT as an adjuvant therapy combined with physical exercise (commonly used in rehabilitation) to mitigate muscle weakness and atrophy after surgical orthopedic procedures.[30]

We would like to thank the authors for the suggestion of new protocols for training programs.

CONCLUSIONS

We understand all the concerns raised by the authors and believe that discussions like this promote progress in science. We disagree with the conclusion of the authors suggesting possible overestimation, bias and deficiencies, and a less-than-strict methodological approach. The results reported[1] were pioneering and were not overestimated or underestimated as demonstrated by the following methods used: (1) criterion (gold) standard methodology, (2) methods already well established in literature, (3) blinded evaluators, and (4) use of placebo therapy. All concerns raised and criticisms of Ferraresi et al.[1] were based on studies with a lack of similarity, using arguments and literature that do not specifically support these concerns. Direct comparisons between studies without comparable methodologies cannot be considered to be reliable, applicable, and acceptable, thus making most of the concerns and criticisms of the authors unproven at best.

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