LACTATE EFFECTS ON MUSCLE REGENERATION IN VIVO AND IN VITRO MODELS: A SYSTEMATIC REVIEW

EFEITO DO LACTATO NA REGENERAÇÃO MUSCULAR EM MODELOS IN VIVO E IN VITRO: UMA REVISÃO SISTEMÁTICA

EL EFECTO DEL LACTATO EN LA REGENERACIÓN MUSCULAR EN MODELOS IN VIVO E IN VITRO: UNA REVISIÓN SISTEMÁTICA

Systematic Review
Revisão Sistemática
Revisión Sistemática

Thiago Ferreira Matos[1]
(Physical Education Professional)
Maria Eduarda Casagrande Carli[1]
(Physical Education Professional)
Frederico Bento de Moraes Junior[1]
(Physical Education Professional)
Valderi Abreu de Lima[1]
(Physical Education Professional)
Jorge Mota[2]
(Physical Education Professional)
Neiva Leite[1,2]
(Physical Education Professional)

1. Federal University of Paraná, Physical Education Department, Curitiba, Paraná, Brazil.
2. University of Porto, Faculty of Sport, Research Centre in Physical Activity, Health and Leisure, Porto, Portugal.

Correspondence:
Maria Eduarda Casagrande Carli
Rua Padre Germano Mayer, 35, apto 902, Curitiba, Paraná, Brazil, 80050-270.
maria96carli@gmail.com.

ABSTRACT

Introduction: Lactate is a substance that, over the years, has been responsible for numerous effects, mainly linked to muscle fatigue, being recently highlighted in systemic processes and muscle regeneration, which still has many points to be clarified, one of them being the participation of lactate in this process. Objective: Verify the effect of lactate on muscle recovery in in vivo and in vitro models. Methods: The search was conducted in five databases (16 June 2022) (PROPERO nº CRD42020209324), using the search words in English and Portuguese: 'lactate', 'lactic acid', 'muscle regeneration', 'MyoD protein', and 'myogenin'. We included experimental and in vitro studies that investigated the use of lactate in relation to muscle (all species and both sexes – males and females). The methodological quality was assessed by a specific questionnaire for in vitro and in vivo studies. Results: Eight articles were selected, of which three studies were found exclusively in vitro, one exclusively in animal models, and four studies with in-vitro models and animal models. Three studies scored overall methodological quality above 90%, another three between 80-90% and two between 70-75%. Both types of studies showed results that may point to the mechanism by which lactate acts in the proliferation and differentiation of myoblasts, either by the action of myogenic regulatory factors (MRFs) or by the actions in the metabolism of these cells. Conclusion: The lactate presence in myoblasts strongly suggests a correlation with the expression of regulatory molecules during muscle regeneration. Future studies must investigate this correlation to advance our understanding of the regulatory mechanisms involved in this process. ***Level of Evidence II; Systematic Review.***

Keywords: Lactic Acid; Regeneration; Myogenic Regulatory Factors; MyoD Protein; Myogenin.

RESUMO

Introdução: O lactato é uma substância que, ao longo dos anos, foi responsabilizado por inúmeros efeitos, principalmente ligado à fadiga muscular, sendo recentemente destacado em processos sistêmicos e na regeneração muscular, a qual ainda possui muitos pontos para serem esclarecidos, dentre eles a participação do lactato neste processo. Objetivo: Realizar uma revisão sistemática sobre o efeito do lactato na recuperação muscular em modelos in vivo e in vitro. Métodos: A pesquisa foi realizada em cinco bancos de dados (16 de junho de 2022) (PROPERO nº CRD42020209324), utilizando as palavras de pesquisa em inglês e português: 'lactato', 'ácido láctico', 'regeneração muscular', 'proteína MyoD' e 'miogenina'. Incluímos estudos experimentais e in vitro que investigaram o uso de lactato em relação ao músculo (todas as espécies e ambos os sexos - machos e fêmeas). A qualidade metodológica foi avaliada por meio de um questionário específico que contempla estudos in vitro e in vivo. Resultados: Foram selecionados oito artigos, dos quais três estudos foram encontrados exclusivamente in vitro, um exclusivamente em modelos animais e quatro estudos com modelos in vitro e modelos animais. Três estudos obtiveram pontuação geral da qualidade metodológica acima de 90%, outros três entre 80-90% e dois entre 70-75%. Ambos os tipos de estudos mostraram resultados que podem apontar o mecanismo pelo qual o lactato atua na proliferação e diferenciação dos mioblastos, seja pela ação de fatores regulatórios miogênicos (MRFs), seja pelas ações no metabolismo dessas células. Conclusão: A presença de lactato nos mioblastos sugere fortemente uma correlação com a expressão de moléculas reguladoras durante a regeneração muscular. Esta correlação deve ser investigada em estudos futuros para avançar a nossa compreensão dos mecanismos reguladores envolvidos neste processo. ***Nível de Evidência II; Revisão Sistemática.***

Descritores: *Ácido Lático; Regeneração; Fatores de Regulação Miogênica; Proteína MyoD; Miogenina.*

RESUMEN

Introducción: El lactato es una sustancia que a lo largo de los años fue responsable por numerosos efectos benéficos o maléficos para nuestro sistema locomotor, relacionados principalmente a la fatiga muscular, siendo recientemente destacado en procesos sistémicos y de regeneración muscular. Sin embargo, es necesario aclarar muchos puntos, uno de ellos es la participación del lactato en este proceso. Objetivo: Realizar una revisión sistemática sobre el efecto del lactato en la recuperación muscular en modelos in vivo e in vitro. Métodos: La búsqueda se realizó en cinco bases de

Associate Editor responsible for the review process: Paulo Zogaib

datos (16 de junio de 2022) (PROPERO nº CRD420209324), utilizando las palabras de búsqueda en inglés y portugués: 'lactate', 'lactic acid', 'muscle regeneration', 'MyoD protein' y 'myogenin'. Se incluyeron estudios experimentales e in vitro que investigaron el uso del lactato en relación con el músculo en todas las especies y ambos sexos. La calidad metodológica se evaluó mediante un cuestionario específico que abarcaba los estudios in vitro e in vivo. Resultados: Fueron seleccionados ocho artículos, los cuales tres estudios se encontraron exclusivamente in vitro, uno exclusivamente en modelos animales y cuatro estudios con modelos in vitro y modelos animales. Tres estudios obtuvieron una calidad metodológica global superior al 90%, otros tres entre el 80-90% y dos entre el 70-75%. Ambos tipos de estudios mostraron resultados que pueden apuntar al mecanismo por lo cual el lactato actúa sobre la proliferación y diferenciación de los mioblastos, o sea a través de la acción de los factores reguladores miogénicos (MRFs) o a través de acciones sobre el metabolismo de estas células. Conclusión: La presencia de lactato en los mioblastos sugiere fuertemente una correlación con la expresión de moléculas reguladoras en la regeneración muscular. Esta correlación debería investigarse en futuros estudios para avanzar en nuestra comprensión de los mecanismos reguladores implicados en este proceso. ***Nivel de Evidencia II; Revisión Sistemática.***

Descriptores: *Ácido Láctico; Regeneración; Factores Reguladores Miogénicos; Proteína MioD; Miogenina.*

DOI: http://dx.doi.org/10.47870/1517-8692202531012021_0325i Article received on 07/23/2021 accepted on 02/16/2024

INTRODUCTION

Lactate went from a disposable product of glycolysis and a promoter of muscle fatigue to an alternative source of energy for mitochondrial respiration, a glycogenic precursor, and a signaling molecule, being even called lactormone by some researchers.[1-3] In muscle regeneration, lactate has been highlighted in the activation and differentiation of muscle satellite cells, mainly in stimulating myogenic regulatory factors, which will form the myofibrils that compose the muscle fiber. In addition to activating satellite cells, it stimulates the formation of myosin heavy chains (MHC), which is a central factor for the proliferation of myofibrils and muscle hypertrophy.[2,4,5]

The process of muscle regeneration induced by lactate is still a matter of debate, with hypotheses demonstrating lactate as a signaling molecule for some anabolic pathways, as the activation of genes responsible for the trigger of satellite muscle cells that have the capacity of differentiation and proliferation to increase the number of muscle fibers.[2,4,6] Thus, understanding the action of lactate on muscle recovery is necessary not only to elucidate the mechanism by which this substance acts on the muscle but also to improve the physical performance of athletes, such as training with vascular occlusion, a training method for athletes who aim for hypertrophy with low loads and that is explained by the mechanisms of action of lactate.[4] Furthermore, through this understanding it is possible to improve the treatment processes of diseases that affect the muscular system, such as the treatment of dystrophies through the action of lactate, due to its action on the balance of follistatin and myostatin.[4]

As studies related to this topic with humans are scarce, our main question is: "Does lactate administration induce muscle regeneration in vivo and in vitro models?" The present study intends to perform a systematic review of scientific articles that evaluated the in-vitro and in vivo effects of the relationship between lactate and muscle recovery, showing the function of signaling molecules for processes that induce the regeneration, differentiation, and proliferation of muscle fibers.

MATERIAL AND METHODS

Design and protocol registration

The data search was carried out on 31 August 2020 and updated on 16 June 2022, based on the recommendations of PRISMA statement[7] and registered on PROSPERO (CRD42020209324).

Databases and search strategy

The data search was carried out in the following databases: WebOfScience, Scopus, ScienceDirect, PubMed, and *Biblioteca Virtual em Saúde* (BVS) in Portuguese and English. From the *Descritores em Ciências da Saúde* (DeCs) and Medical Subject Headings (MeSH), descriptors in Portuguese and English were selected for the present research: lactates (*lactato*), lactic acid (ácido lático), muscle regeneration (*regeneração muscular*), MyoD protein (*proteína MyoD*), myogenin (*miogenina*). To search for the articles, combinations of the cited descriptors were used, both in English and Portuguese according to the database, using the Boolean operators AND and OR, the terms being grouped as follows: ((lactato OR "ácido lático") AND ("regeneração muscular" OR "proteína MyoD" OR miogenina)), (("lactic acid" OR lactates) AND ("muscle regeneration" OR "myoD protein" OR myogenin)). The complete search strategy is present in the supplementary material.

For inclusion of the articles, the criteria adopted were: (I) experimental studies were included in animal models that investigated the use of lactate in relation to muscle (all species, both sexes – male and female); (II) in vitro studies combined with in vivo studies were included if they used the same species and sex to investigate the use of lactate in muscle regeneration; (III) in vitro studies that investigated the use of lactate in relation to muscle regeneration. No restrictions were applied in relation to the route of administration, dosage, duration of the study, or frequency of dosage. For the exclusion of the articles, the criteria adopted were: (I) experimental studies with human patients only; (II) if lactate and its role are not the focus of the study (III) control group not included or vehicle/control not defined (IV) case studies.

The search was performed by two researchers independently (TFM e MECC), with the selection of articles according to the predetermined inclusion and exclusion criteria. In case of disagreement about the selection of a title, abstract or article, a third researcher was consulted (FBMJ).

Data Extraction and Methodological Quality Score

The extraction data was of a qualitative character, being extracted: (I) the author's name and year of publication; (II) the country/place of the research, (III) the characteristic and quantity of the sample (cell culture and animal species); (IV) the variables analyzed by the study, especially those related to the lactate effects, myogenic regulatory factors (MRF) expression, mRNAs expression, lactate dehydrogenase expression, muscle growth factors, and satellite cell activity, or others variables of interest that would complement our analyses; (V) measurement methods and administration protocols, such as lactate concentrations used and exposure time; (VI) lastly, results obtained according to the variables analyzed were extracted. The data were extracted by two independent researchers (TFM and MECC) and tabulated for analysis.

Subsequently, the selected studies were read in full and evaluated regarding their methodological quality using the questionnaire developed by Carneiro et al. (2020)[8], which was based on existing guidelines for articles, journal checklists, and previous studies on the quality of reports[8]. This questionnaire was chosen based on the fact there is no consensus regarding the methodological assessment of in vitro studies and because most of the selected studies contain a sample with this biological model.

The questions cover five areas of study classification, risk of bias (e.g. blinding, conflict of interest reporting) with four questions in total; drugs and reagents (e.g. antibody validation, source of reagents) addressing four questions; data presentation (e.g. summary and measures of variation, identifiable groups, the definition of symbols used) with eight questions; data analysis (e.g. statistical tests used, exact p values) with six questions; and details on the biological model according to each study (e.g. culture conditions, animal species, and strains, ethical requirements), with four questions for in vitro studies and 12 questions for vertebrate animal studies. In the case of studies containing a mixed sample (in vivo and in vitro, all 16 questions were evaluated). Each question scored from 0 to 1, with 1 representing that the question was contemplated, 0.5 partially contemplated, and 0 not contemplated. In some cases, there was the option of non-applicability. The detailed questionnaire is in the supplementary materials.

Overall scores for the studies were defined by the percentage of items reported for each article, using the total number of applicable questions - defined by both the biological model category and the number of questions. The overall report scores considered only the questions in the first five sections of the questionnaire, while the specific scores considered the section for the corresponding biological model of the outcome under review.

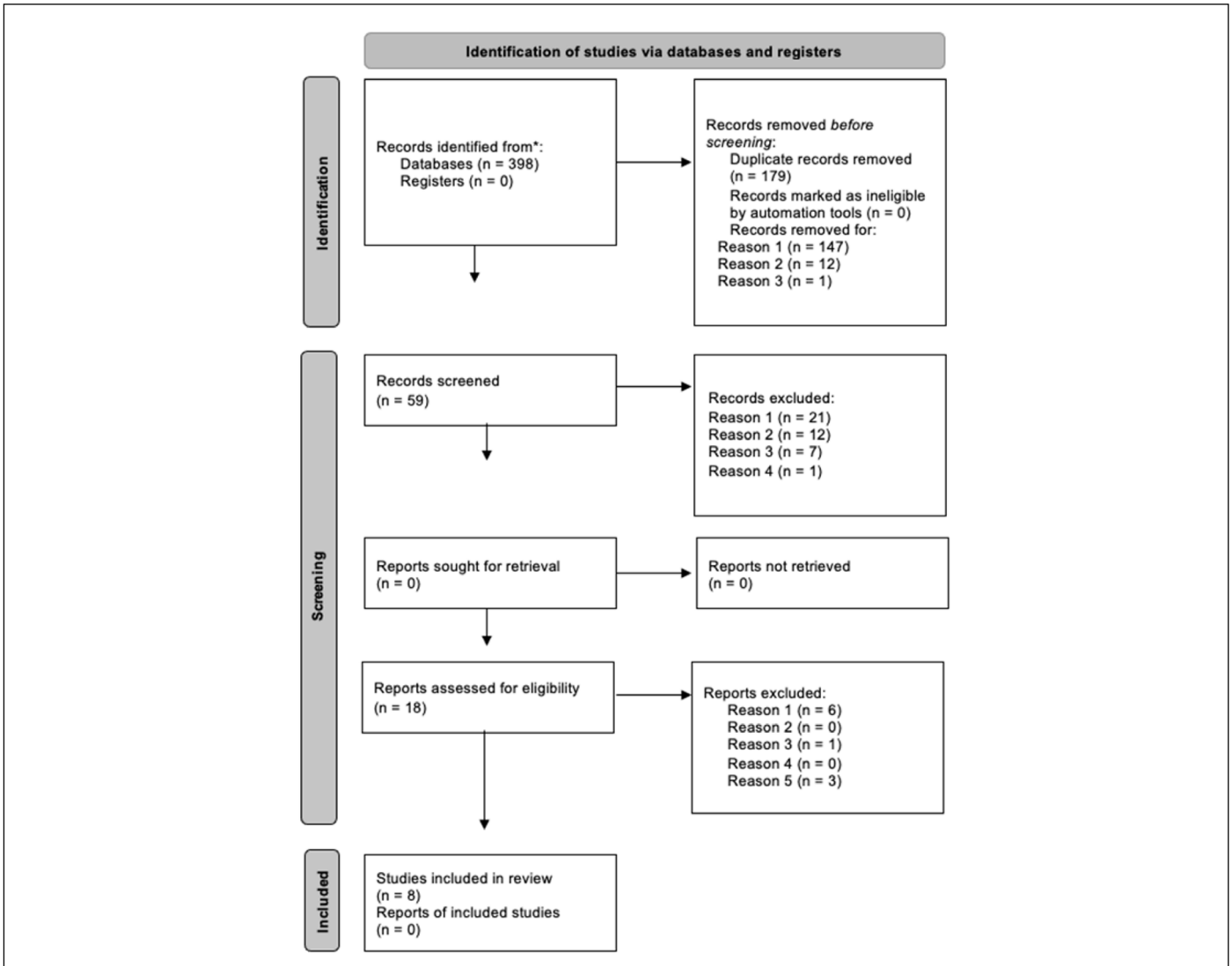
RESULTS

Study selection

A total of 398 articles were found in all databases. After reviewing repeated studies, 219 remained. After screening for title and abstract, 18 articles were selected for full-text reading. Finally, for qualitative synthesis, eight articles were selected for this review (Figure 1).

Assessment of Methodological Quality

The complete methodological quality assessment, i.e., with all questions and score for each question, is present in the supplementary material. Table 1 shows the overall score of the studies by the percentage of items reported for each article. Of the eight studies, three obtained



Note: Reason 1 = studies not related to the theme (n = 174). Reason 2 = books, book chapters, monographs, dissertations, theses, review articles, case studies, abstracts, letters to the editor, editorial and consensus (n = 24). Reason 3 = studies that did not correlate the variables of interest (n = 9). Reason 4 = study that did not provide an abstract (n = 1). Reason 5 = lactate and its role are not the study's focus (n = 3).

Figure 1. Flowchart with the stages of study selection.

average scores above 90%[4,6,9], three reached between 80-90%[10–12] and only two had averages around 70-75%[13,14]. Most studies scored lower in the risk of bias and data presentation areas.

Characterization

The year of publication of the studies ranged from 1998[13] to 2019.[4,15] Most studies were done in Asia, four, [4,6,10,15] two in Europe[11,13] and two in North America. [9,14]

The characteristics of the samples included are clearly described in most of the studies, and in six of them[4,6,10,11,14–16] the cells used were cells of C2C12 rat myoblasts, a cell line that were immortalized and are now commercialized for use in studies with the purpose of evaluate changes and muscle formation. In one study,[13] muscle cells of the hind limb of New Zealand white rabbit embryos were used. In addition to C2C12 cells, three studies used cells from mice. [4,6,10] And in the only study exclusively done with an animal model, male C57 / BL6 rats with lesions induced by bupivacaine were used.[9] The characteristics and main results of the studies are shown in Table 2.

Of the eight studies included in this review, six [4,6,10,11,14,15] observed the role of lactate for markers of muscle regeneration and one[13] focused only on how differentiation and proliferation of new muscle fibers occurs, while another[9] observed the appearance of lactate transporters. Table 3 shows the comparisons between the results obtained by the studies and the differences in the methodology regarding the MRFs. Willkomm et al. (2017)[11] used 2 types of solutions in his cell samples one called the Proliferation (PM) solution and a Differentiation (DM) solution:

DM = 1% penicillin/streptomycin, 4 mM glutamine, 1.5 g/L sodium bicarbonate, 1 mM sodium pyruvate, and 4% horse serum PM = 1%

Table 1. Quality percentage of selected studies.

Study	Title / abstract	Risk of bias	Drugs e reagents	Data presentation	Data analysis	Specific criteria*	Mean %
Barjot et al. (1998)[21]	100%	25%	100%	75%	17%	100%	70%
Washington et al. (2013)[9]	100%	75%	100%	88%	100%	83%	91%
Oishi et al. (2016)[10]	100%	25%	100%	100%	100%	88%	86%
Acharya et al. (2017)[14]	100%	50%	100%	50%	67%	75%	74%
Willkomm et al. (2017)[11]	100%	50%	75%	88%	67%	100%	80%
Tsukamoto et al. (2018)[22]	100%	50%	100%	100%	100%	94%	91%
Oh et al. (2019)[15]	100%	50%	100%	67%	67%	100%	81%
Ohno et al. (2019)[4]	100%	50%	100%	100%	100%	100%	92%

Note: the percentages refer to how well that study contemplated the criteria. * specific score considered the section for the corresponding biological model of the outcome under analysis.

Table 2. Description of selected studies.

Author / year / Location	Sample	Analyzed variables	Main results
barjot et al. (1998)[21] France	Hindlimb muscle cells of New Zealand white rabbit embryos	Desmin Reactivity. Expression of MRFs mRNAs. Expression of LDH genes. Expression of MyHC genes	Desmin: changes its orientation. Myf5, MyoD, myogenin and MRF4 mRNA: present since day 1. LDH activity: increased during the proliferation and differentiation phase. Slow adult MyHC cells: detected in some myofibers. Fast adult MyHC cells: not detected
Washington et al. (2013)[9] United States of America	Male rats C57/BL6	Expression of MRF and growth factors, MCT1 and MCT4, of LDH-A and LDH-B	IGF-1: increased 5x. MyoD: increased 4x, 3 days after the muscle's injury. MCT1 and MCT4: decreased 3 days after injury. LDH-A: increased 71%. LDH-B: decreased 53%
Oishi et al. (2016)[10] Japan	C2C12 cells and male F344/DuCrlCrlj mice	Effects of LA, CA, or LC compound on satellite cell activity, cell cycle and muscle anabolic signals. Effects of training and LC on muscle weight, DNA content, myofibrillar protein concentration, satellite cell activity and expression of Fst and Mstn	Activated satellite cells shown higher MyoD expression - LC significantly increased MyoD protein levels when compared to the LA group. Treatment with LC increased Ki67. LA and LC showed a greater presence of Fst than the control without changing Mstn
Acharya et al. (2017)[14] United States of America	C2C12 cells	Antioxidant effect of MF. Protective effect of MF against oxidative stress - measuring the expression of MRFs. Effect of MF on Nrf2 expression	The efficacy of MF was similar to AA and MY at concentrations 100 µM. 10 µM. LA (20 mM) reduced cell viability, but 25 and 50 µM MF recovered it. MyoD and Myogenin had a greater expression when combined LA with MF or AA at 50µM. Effect dose dependent on MF and Nrf2 expression
Willkomm et al. (2017)[11] Germany	C2C12 cells and 15 healthy men (age: 23±3 years; height: 180±6cm; weight: 76.2±8.3 kg)	LA and protein p38. LA and H3K4me3. LA and Myf5, myogenin and MyHC	20mM LA for 24h = p38 disappeared and H3K4me3 was inhibited until it disappeared. The 20mM solution also suppressed mRNA expression for MRFs
Tsukamoto et al. (2018)[22] Japan	C2C12 cells and male ICR mice	LA as a promoter of myoblast differentiation / LA improving the transcription of MyoD and MyHC genes / LA increases the transcription of MyHC isoforms in a dose-dependent manner	5 days with 10mM of LA accelerated the myoblasts fusion and myotubes hypertrophy. Myf5 and myogenin levels did not change in the presence of LA, but Myh4 had an increase, which increased the amount of MyHC present in the formed myotubes. MyoD was also influenced by LA. LA above 6 mM directly affects the expression of Myh4
Oh et al. (2019)[15] South Korea	C2C12 cells	Initial responses of AMPK signaling to LA / Initial responses of myogenic proteins and Akt-FOXO3a pathway to LA / Late responses of AMPK signaling to LA / Inhibition of Akt-mediated myogenic pathways by LA overload for 24h	8mM LA for 1h had no significant difference in p-AMPK. LA in myogenic protein levels and the Akt-FOXO3a/LA pathway (8mM) for 24h significantly increased the p-AMPK. LA (8 mM) for 24 h inhibited p-Akt levels, MyoD and MyHC
Ohno et al. (2019)[4] Japan	C2C12 cells and male C57BL/6J mice	Effects of LA on skeletal muscle for hypertrophy, regeneration and myotubes formation	Myotubes treated with LA (20mM) had a larger diameter, greater length and more myonuclei when compared to the control group

Note: MRFs = myogenic regulatory factors, LDH = Lactate Dehydrogenase, MyHC = Myosin Heavy Chain, MyoD = myoblast determination protein 1, MCT = monocarboxylate transporter, IGF = insulin growth factor, LA = lactate, CA = caffeine, LC = lactate + caffeine, Fst = follistatin, Mstn = myostatin, Nrf2 = nuclear factor erythroid 2, MF = maniflavone, AA = ascorbic acid, MY = myricetin.

penicillin-streptomycin, 4 mM glutamine, 1.5 g/L sodium bicarbonate, 1 mM sodium pyruvate, and 20% fetal calf serum.

As a way of illustrating the various methodological differences highlighted in Table 3, Figure 2 was generated. Some methodological aspects had to be ignored in group studies relating only days of exposure and lactate concentration to the MRFs (only the MyoD protein and the Myogenin were chosen, given that they were the proteins that most appeared in the studies). The study by Oishi et. al. (2016)[10] was taken from this illustration because the study exposure time is less than one day (the sample was exposed for only 6 hours to lactate).

DISCUSSION

The first important finding in the present study is that our search resulted in the selection of eight studies with in-vitro samples and animal models, demonstrating the function of lactate as a signaling molecule for processes that induce the regeneration and proliferation of muscle fibers. The six studies that tested lactate as a promoter of muscle regeneration used different concentrations and exposure times, a factor that makes it difficult to compare the results between researchers. Differences are highlighted in the conduct of studies that may have interfered in the outcomes, in which three studies placed their cell culture exposed to a sodium lactate solution at a concentration of 20mM for five days[4,11,14], one used 10mM for five days,[6] another study used 8mM for 24 hours[15] and finally, one study used 10mM for 6 hours.[10]

Regarding the expression of MRFs, the results observed were contradictory. While five studies[6,9,10,14] found increased expression of these proteins (MyoD and myogenin), with one of them [4] not declaring which protein presented such a result, two studies[11,15] noticed a decrease (MyoD, myogenin, and Myf5), and finally a study[13] noted that during the first seven days of embryonic muscle cell culture, MRFs (MyoD, Myf5, and myogenin) were elevated on day one and decreased steadily until day seven (rising during the proliferation period and decreasing in the periods of differentiation and maturation of myotubes). On the other hand, MRF4 was low on day one and increased until day seven, at the same time as there was a linear increase in LDH expression during proliferation and differentiation of myoblasts. It is worth mentioning here that the study by Washington et al. (2013)[9] observed an increase in LDH-A (converts pyruvate into lactate) by 71% and LDH-B (converts lactate into pyruvate) decreased 53%.

The lactate concentrations used, the exposure times, and the normalizations made with different proteins make a direct comparison of the obtained results ineffective. Respecting the individualities of each study, two studies can be paired,[6,14] due to the similarity of exposure time and the normalization to measure the variation - MRF / β-Actin. Notably, despite having only half of the lactate concentration compared to Acharya et al. (2017),[14] Tsukamoto et al. (2018)[6] obtained the same level of success as the other study in protein Myo. These results suggest that the variation is dependent and directly related to the amount of lactate used in the experiments. The sustained elevation of lactate would assist in the myogenic differentiation as well as in the hypertrophy of the muscle fiber and the suggested mechanism would be by the increase in the expression of MyoD facilitated by the lactate.[6]

Oishi et al. (2016)[10] found that muscle mass and the number of myonuclei can be increased efficiently by administering the lactate+caffeine compound, even during low-intensity exercises. This is achieved by increasing the activity of satellite cells and the presence of anabolic markers in the muscle. In contrast, Oh et al. (2019)[15] discovered that a 24-hour lactate overload inhibits myogenic pathways by activating AMPK and suppressing Akt, which decreases the levels of myogenic factors and increases the levels of the atrophic factor MAFbx. Additionally, Ohno et al. (2019)[4] found that oral lactate administration is associated with hypertrophy and muscle regeneration in rats, and extracellular lactate may contribute to the regulation of skeletal muscle plasticity. It is worth mentioning that the MyoD protein is related to the proliferation of myoblasts while Myogenin is linked to the differentiation of these cells.[17,18] However, there is difficulty of establishing a causal relationship between the presence of lactate and muscle regeneration, due to the lack of standards in the methodology of the studies included in this review.

Another problem identified in this review was the focus of the selected studies. Although they present results related to muscle regeneration, the main objective of the studies was not really to evaluate lactate as a promoter of this regeneration. One study aimed to show manniflavone as a more viable substance than ascorbic acid as a cellular antioxidant,[14] another study aimed to show how the development of

Table 3. Lactate and MRFs.

Study	[ ] of lactate (mM)	Days of exposure	Reason	OBS	Values
Barjot et.al. (1998)[21]	N/A	7	MRF / S26	S26, Ribosomal protein	Myf5 = -4,0 MyoD = -3,5 Myog = -0,4 Myf6 = +3,0 LDH = +1,0 LDHactivity = +1,8
Washington et. al (2013)[9]	N/A	3	MRF/18s mRNA Abundance	18s mRNA RNA ribossomal	MyoD = +2,5 LDH-A = +0,7 LDH-B = -0,5
Oishi et.al. (2016)[10]	10	6 horas	Arbitrary Values	Foi medida a variação	MyoD = +0,4 (LC) or +0,2 (LA) Myog = +0,2 (LC) or +0,3 (LA)
Acharya et.al. (2017)[14]	20	5	MRF / β-Actina	β-Actina, proteína de formação do músculo	MyoD = +0,7 (only LA) or +1,4 (LA + MF50)
Willkomm et.al. (2017)[11]	20	5	x-fold increase	Measured the variation	Myf5 = -0,30 Myog = -1,2 MHC1 = -1,0 MHC2 = -1,1
Tsukamoto et.al. (2018)[22]	10	5	MRF / β-Actin	β-Actin Muscle-forming protein	MyoD = +0,3 Myog = -0,2 MHC = +0,2
Oh et.al. (2019)[15]	8	1	MRF / α-Actin	α-Actin Muscle-forming protein	MyoD = -0,8 Myog = -0,5
Ohno et.al. (2019)[4]	20	5	-/-	MRFs not evaluated	N/A

Note: [ ] = concentration; OBS = observation, MRFs = myogenic regulatory factors, LDH = lactate dehydrogenase, LA = lactate, LC = lactate + caffeine, MF50 = 50mM maniflavone.

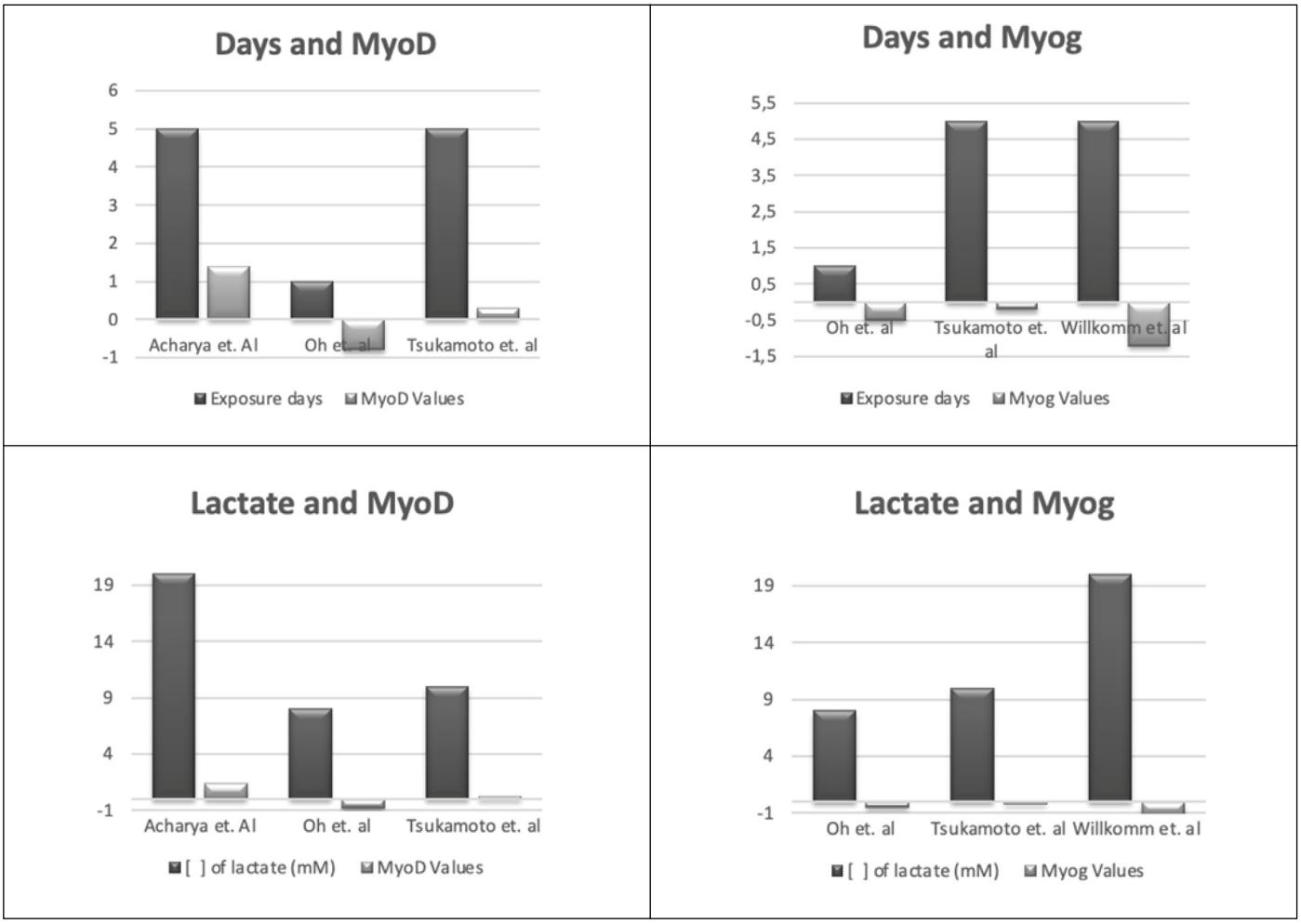


Figure 2. Exposure days, lactate concentration and MRFs ratio.

muscle in fetuses of rabbits happens,[13] one looked at the expression of monocarboxylate transporters in muscle regeneration,[9] one focused on combining caffeine and lactate as a method for increasing the activity of muscle satellite cells,[10] two sought to present lactate as a promoter of muscle regeneration[4,6] and two other studies attempted to present lactate as an inhibitor or an impediment to muscle regeneration.[11,15]

A recent study proposes a new approach to muscle regeneration,[19] in which the focus is on succinate. The author presents a model in which exercise would lower intracellular pH during lactate production. This decrease would make it possible to protonate the succinate by transforming the molecule from a dicarboxylate to a monocarboxylate, which allows this molecule to be exported via the monocarboxylate receptors (MCT1 and MCT4). Thus, in the extracellular matrix, the succinate would bind to non-muscle cells in its SUCNR1 receptor (a receptor coupled to the G protein), which activates studied cell remodeling pathways such as PKA and MAPK. Through this activation, the succinate would be responsible for acting in the neuronal and interstitial restructuring of the muscle cell. There may be a parallel effect that happens when intracellular pH decreases, and lactate accumulates in the cell. According to this review, lactate accumulation is associated with a greater expression of MRFs [6,9,10,14], which are responsible for other myogenic pathways. Therefore, this factor cannot be ruled out as a potential contributor to the observed effects.

Although Brooks (2020)[3] proposed lactate as the protagonist of numerous functions in human metabolism, the author did not suggest it as a possible promoter of muscle regeneration. However, Reddy et al. (2020)[19] presented succinate in their study and established a direct connection between the lactate molecule and the activation of pathways that lead to the proliferation and differentiation of myoblasts. Regarding the clinical use of lactate, that is, for the treatment of diseases related to muscle degeneration such as dystrophies, the mechanism by which lactate can be an alternative treatment still needs to be further tested. Although some results in the size and weight of the fibers may indicate a course of action[4], it is necessary to test whether the correlation of the presence of lactate in the muscle stimulated myogenesis via an increase in follistatin, or an increase in MRFs or if there is any other molecule participating in this process. Explaining this relationship would also serve to explain the reason for positive results for muscle hypertrophy in training with vascular occlusion, in which the restriction of oxygen in the muscle increases the local lactate concentration and imprisons the lactate for longer periods in the muscle.[4,20]

As a limitation of the present study, so far, there is only a correlation between the presence of lactate and the expression of myogenic regulatory factors. It is not yet possible to establish whether this correlation is positive or negative, much less, whether it is a strong or weak correlation in humans, it was only possible to observe that the presence of lactate in experiments with cells and some animal models alter the way these molecules are expressed. In addition, most studies presented a concerning risk of bias. Still, we emphasize that the report's methodological quality evaluation was based on a previous study, but not with a specific tool for these types of experiments. Therefore, we used an unweighted score as a parameter.

For future studies, a similar methodology must be adopted so that the results can be compared efficiently, and that the main objective of the study is to evaluate lactate as the promoter of muscle regeneration. Taking as a starting point the study by Reddy et al. (2020),[19] it is necessary to determine whether lactate plays a leading role in the activation

of myoblasts or if it is only a molecule that marks cellular stress (in the context of muscle regeneration) and other molecules of our metabolism would be the ones that truly activate the muscle regeneration process, being lactate only an indicator and not the promoter.[19]

CONCLUSION

This review showed that although studies still have many methodological differences, lactate could be a molecule that is participating in the myogenesis process because its presence in muscle cells is related to the appearance of other proteins that are intrinsically related to the proliferation and differentiation of myoblasts. The relationship between the appearance of lactate inside myoblasts and the expression of regulatory molecules in the muscle regeneration process may not yet be a causal process; however, there may be a correlation between both that needs to be tested.

ACKNOWLEDGEMENTS

The authors are grateful for the financial support provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001, *Programa* Professor *Sênior – Federal University of Paraná*, Fundação para a ciência e tecnologia [FCT: SFRH/BSAB/142983/2018 and UID/DTP/00617/2019], Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq - PQ grants), Programa Pesquisa Para o SUS (PPSUS – edital - CP 01/2016) via Fundação Araucária de Apoio ao Desenvolvimento Cientifico e Tecnológico do Estado do Paraná (Decit/MS) and Secretaria da Saúde do Estado do Paraná (SESA-PR).

All authors declare no potential conflict of interest related to this article

AUTHORS' CONTRIBUTIONS: Each author contributed individually and significantly to the development of the manuscript. TFM: conception, writing, acquisition, analysis, and interpretation of data for the paper; MECC: conception, writing, acquisition, analysis, interpretation of data for the paper, and review. FBMJ: conception, design, and critical revision of the manuscript. JM: critical revision of the manuscript. NL: conception, design, writing, critical review, and final approval of the manuscript.

REFERENCES

1. Philp A, Macdonald AL, Watt PW. Lactate - A signal coordinating cell and systemic function. J Exp Biol. 2005;208(24):4561-75. doi:10.1242/jeb.01961.
2. Nalbandian M, Takeda M. Lactate as a signaling molecule that regulates exercise-induced adaptations. Biology (Basel). 2016;5(4):38. doi:10.3390/biology5040038.
3. Brooks GA. Lactate as a fulcrum of metabolism. Redox Biol. 2020;35:101454. doi:10.1016/j.redox.2020.101454.
4. Ohno Y, Ando K, Ito T, Suda Y, Matsui Y, Oyama A, et al. Lactate stimulates a potential for hypertrophy and regeneration of mouse skeletal muscle. Nutrients. 2019;11(4):869. doi:10.3390/nu11040869.
5. Hashimoto T, Tsukamoto H, Takenaka S, Olesen ND, Petersen LG, Sørensen H, et al. Maintained exercise-enhanced brain executive function related to cerebral lactate metabolism in men. FASEB J. 2018;32(3):1417-27. doi:10.1096/fj.201700381RR.
6. Tsukamoto S, Shibasaki A, Naka A, Saito H, Iida K. Lactate Promotes Myoblast Differentiation and Myotube Hypertrophy via a Pathway Involving MyoD In Vitro and Enhances Muscle Regeneration In Vivo. Int J Mol Sci. 2018;19(11):3649. doi:10.3390/ijms19113649.
7. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ. 2021;372:n71. doi:10.1136/bmj.n71.
8. Carneiro CFD, Queiroz VGS, Moulin TC, Carvalho CAM, Haas CB, Rayêe D, et al. Comparing quality of reporting between preprints and peer-reviewed articles in the biomedical literature. Res Integr Peer Rev. 2020;5(1):16. doi:10.1186/s41073-020-00101-3.
9. Washington TA, Brown L, Smith DA, Davis G, Baum J, Bottje W. Monocarboxylate transporter expression at the onset of skeletal muscle regeneration. Physiol Rep. 2013;1(4):e00075. doi:10.1002/phy2.75.
10. Oishi Y, Tsukamoto H, Yokokawa T, Hirotau K, Shimazu M, Uchida K, et al. Mixed lactate and caffeine compound increases satellite cell activity and anabolic signals for muscle hypertrophy. J Appl Physiol. 2015;118(6):742-9. doi:10.1152/japplphysiol.00054.2014.
11. Willkomm L, Gehlert S, Jacko D, Schiffer T, Bloch W. P38 MAPK activation and H3K4 trimethy lation is decreased by lactate in vitro and high intensity resistance training in human skeletal muscle. PLoS One. 2017;12(5):e0176609. doi:10.1371/journal.pone.0176609.
12. Oh MS, Phelps KR, Traube M, Barbosa-Saldivar JL, Boxhill C, Carroll HJ. D-Lactic Acidosis in a Man with the Short-Bowel Syndrome. N Engl J Med. 1979;301(5):249-52.
13. Barjot C, Laplace-Marieze V, Gannoun-Zaki L, Mckoy G, Briand M, Vigneron P, et al. Expression of lactate dehydrogenase, myosin heavy chain and myogenic regulatory factor genes in rabbit embryonic muscle cell cultures. J Muscle Res Cell Motil. 1998;19(4):343-51. doi:10.1023/A:1005389418903.
14. Acharya S, Stark TD, Oh ST, Jeon S, Pak SC, Kim M, et al. (2R,3S,2″R,3″R)-Manniflavanone Protects Proliferating Skeletal Muscle Cells against Oxidative Stress and Stimulates Myotube Formation. J Agric Food Chem. 2017;65(18):3636-46. doi:10.1021/acs.jafc.6b05161.
15. Oh SSJ, Kim S, Moon S, Park DH, Kang JH. Lactate overload inhibits myogenic activity in C2C12 myotubes. Open Life Sci. 2019;14(1):29-37. doi:10.1515/biol-2019-0004.
16. Frawley K, Greenwald G, Rogers RR, Petrella JK, Marshall MR. Effects of Prior Fasting on Fat Oxidation during Resistance Exercise. Int J Exerc Sci. 2018;11(2):827-33.
17. Hernández-Hernández JM, García-González EG, Brun CE, Rudnicki MA. The myogenic regulatory factors, determinants of muscle development, cell identity and regeneration. Semin Cell Dev Biol. 2017;72:10-8. doi:10.1016/j.semcdb.2017.11.010.
18. Engert JC, Berglund EB, Rosenthal N. Proliferation precedes differentiation in IGF-I-stimulated myogenesis. J Cell Biol. 1996;135(2):431-40. doi:10.1083/jcb.135.2.431.
19. Reddy A, Bozi LHM, Yaghi OK, Mills EL, Xiao H, Nicholson HE, et al. pH-Gated Succinate Secretion Regulates Muscle Remodeling in Response to Exercise. Cell. 2020;183(1):62-75.e17. doi:10.1016/j.cell.2020.08.039.
20. Asfour HA, Allouh MZ, Said RS. Myogenic regulatory factors: The orchestrators of myogenesis after 30 years of discovery. Exp Biol Med (Maywood). 2018;243(2):118-28. doi:10.1177/1535370217749494.