RAMB
Revista da Associação Médica Brasileira

AMB
Associação Médica Brasileira

Volume 63
Número 2
February 2017
ISSN 0104-4230
ISSN 1806-9282 (*On-line*)

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JOURNAL OF THE BRAZILIAN MEDICAL ASSOCIATION

AMB Associação Médica Brasileira

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Associação Médica Brasileira

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Editor-in-chief: Carlos V. Serrano Jr.
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Website: www.ramb.org.br

The norms for publication are available on the website www.ramb.org.br

The Journal of the Brazilian Medical Association is affiliated to the ANATEC and indexed in Medline, SciELO, Science Citation Index Expanded, Journal Citation Reports, Index Copernicus, Lilacs, and Qualis B2 Capes databases, and licensed by Creative Commons®. Registered in the 1st Office of Registration of Deeds and Documents of São Paulo under n. 1.083, Book B, n. 2.

The Journal of the Brazilian Medical Association is an official publication of the Associação Médica Brasileira (AMB), distributed exclusively to the medical community in Brazil and Latin America.

Manole Publisher

Authorizing editor: Walter Luiz Coutinho
Editor: Karin Gutz Inglez
Publishing production: Fernanda Quinta and Cristiana Gonzaga S. Corrêa
English version: Graziella Risolia Gallo
Cover: Rafael Zemantauskas
Graphic design: Sopros Design
Layout: Lira Editorial

SUMMARY

Volume 63 – Número 2 – February 2017
ISSN 0104-4230 – ISSN 1806-9282 (*On-line*)

SECTIONS

ORIGINAL ARTICLES

REVIEW ARTICLES

Radiotherapy and the SUS: A collapse foretold

Radioterapia e SUS: o colapso anunciado

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Article received: 11/29/2016
Accepted for publication: 12/19/2016

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http://dx.doi.org/10.1590/1806-9282.63.02.93

Radiotherapy, along with surgery and chemotherapy, is one of the pillars of the treatment of cancer patients. However, radiotherapy in Brazil is currently in a critical situation, especially with regard to the care of patients assisted by the Unified Health System (SUS, in the Portuguese acronym). The main problems that contribute to this dicey scenario are related to inadequate remuneration and poor installed capacity, both from the point of view of the number of devices and their geographical distribution.[1]

It is estimated that around 60% of all cancer patients will require radiotherapy at some stage of their treatment.[2] Thus, based on the rounded calculation of 600,000 patients diagnosed with cancer in Brazil in 2015,[3] 360,000 should have received radiotherapy in that year. Considering that approximately 80% of the demand comes from the public health network, it is estimated that 288,000 individuals should have been treated through the SUS. Nevertheless, data from the Ministry of Health indicate that only 145,180 patients received radiotherapy covered by the SUS.[4] In addition, the World Health Organization (WHO) recommends that there should be one radiotherapy device for every 300,000 inhabitants.[2] In Brazil, the need would be 683 devices in operation for the demand to be met. If we also consider that 80% of the patients are assisted by the SUS, we conclude that there is a need for an installed capacity of 546 devices totally dedicated to the public health system. But according to the Ministry of Health, there are currently only 269 radiotherapy devices that serve social security patients and they are often not exclusively dedicated to public care.[1] Again, the number falls far short of the needs.

Another important aspect of this problem is the poor distribution of radiotherapy devices throughout the country. In the South and Southeast regions, installed capacity exceeds 60% of the demand, while in the North, Northeast and Midwest, it is less than 40%.[1]

In 2011, the Federal Audit Court[5] audited a report on care for cancer patients in Brazil. In this report, the lack of radiotherapy was evident and the precarious situation of a large number of patients treated by the SUS, who waited on average 113.4 days between the date of diagnosis and the beginning of radiotherapy, became clear. Five years after the report, nothing concrete happened to increase the offer of radiotherapy to the population.

Although it is recognized that the public health system has a serious management problem and that by rationalizing the resources allocated to it we could have better results, the fact that the radiotherapy remuneration values have been frozen since August 2010 is decisive for the current alarming scenario. Maintenance of equipment, as well as the purchase of spare parts and new machines are charged in US dollars, and the dollar exchange price has been valued more than 115% since the freezing of the remuneration. All other expenses also increased by at least 50%, leading to the insolvency of all services covered by the SUS that do not receive supplementary funds.

In 2012, the Ministry of Health issued Ordinance no. 931 (May 10, 2012),[6] which provided for the acquisition by the Federal Government of 80 machines to be used by the SUS network. This is a well-intentioned plan, including the installation of a radiotherapy machine factory in Brazil, and the implementation of a training center for professionals. However, due to bureaucratic and operational problems, only two of the machines have been installed so far.

We understand that the economic situation of the country is delicate and that seeking new resources is not a simple task, but if nothing is concretely done, the already installed collapse of public radiotherapy will become

substantially worse. Meanwhile, a few thousand patients are waiting in line, hoping that death will not be their announced sentence.

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Treatment of benign prostatic hyperplasia

Tratamento da hiperplasia prostática benigna

Authorship: Brazilian Society of Urology (SBU)
Participants: Ricardo Vita Nunes[1], João Manzano[1], José Carlos Truzzi[1], Aguinaldo Nardi[1], Antonio Silvinato[1], Wanderley Marques Bernardo[2]
Final draft: August 7, 2016

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http://dx.doi.org/10.1590/1806-9282.63.02.95

The Guidelines Project, an initiative of the Brazilian Medical Association, aims to combine information from the medical field in order to standardize procedures to assist the reasoning and decision-making of doctors.
The information provided through this project must be assessed and criticized by the physician responsible for the conduct that will be adopted, depending on the conditions and the clinical status of each patient.

Grades of recommendation and levels of evidence

A: Experimental or observational studies of higher consistency.
B: Experimental or observational studies of lower consistency.
C: Cases reports (non-controlled studies).
D: Opinion without critical evaluation, based on consensus, physiological studies or animal models.

Objective

To advise physicians on the most indicated therapeutic possibilities that can improve the symptoms of the urinary tract and the quality of life of the patient, as well as prevent complications related to the evolution of benign prostatic hyperplasia.

Introduction

A significant proportion of men with benign prostatic hyperplasia (BPH) do not require treatment. Such observation does not necessarily imply the absence of treatment. We can achieve improvement of lower urinary tract symptoms (LUTS) by adopting some non-pharmacological measures, such as reducing nocturnal water intake, reducing caffeine and alcohol consumption, and avoiding the use of decongestants and antihistamines. Men with mild or moderate urinary tract symptoms that have minimal impact on quality of life are candidates for active monitoring requiring annual reevaluation.[1] (**D**)

What are the therapeutic possibilities for medicines that improve urinary symptoms and prevent complications related to the progression of benign prostatic hyperplasia?

Search strategy
(Hyperplasia OR Benign Prostatic Hypertrophy OR BPH OR Prostatic Hyperplasia) AND (Adrenergic alpha-1 Receptor Antagonists OR Adrenergic alpha-2 Receptor Antagonists OR Adrenergic alpha-Antagonist OR Adrenergic alpha Blockers OR 5-alpha Reductase Inhibitors OR 5ARIs OR Muscarinic Antagonists OR Antimuscarinics OR Phosphodiesterase 5 Inhibitor* OR Inhibitors, PDE-5 OR PDE5) AND (Therapy/Narrow[filter] OR systematic[sb])
1st selection: 717
Main exclusion factors: Non-epidemiological studies, studies not related to PICO, duplicate studies, weak evidence strength (Oxford and GRADE), articles in languages other than Portuguese, English or Spanish
2nd selection: 32

Phytotherapy

Due to the lack of scientific evidence, the systematic use of herbal medicines, such as Saw palmetto (*Serenoa repens*) extract, to treat BPH-associated LUTS (BPH-LUTS) is not recommended.[2] (**A**) The PROCOMB trial showed that the combination therapy of tamsulosin and phytotherapy based on *Serenoa repens* plus selenium and lycopene was more effective in improving International Prostate Symptom Score (I-PSS) than either therapy alone

(18.2% using combined therapy vs. tamsulosin alone [p<0.05] and vs. phytotherapy alone [p<0.05]).[3] (**A**)

Alpha-blockers

These are involved in the regulation of smooth muscle tone of prostate and bladder neck, and are critical mediators of lower urinary tract symptoms and the pathophysiology of BPH-LUTS. For this reason, alpha-blockers are first-line drugs in the treatment of BPH symptoms.[3] (**A**) Alpha-blockers currently available: doxazosin, tamsulosin, alfuzosin, terazosin and silodosin, the last one not available in Brazil. Although there are small differences between alpha-blockers, they are all equally effective and lead to a 4- to 6-point objective drop in I-PSS, enough for most patients to report significant improvement in symptoms. These are considered to be very efficient drugs as monotherapy in the treatment of BPH-LUTS.[4,5] (**A**) Patients candidate for monotherapy with alpha-blockers are mainly those with moderate to severe LUTS and with an impact on quality of life. Symptomatic improvement is perceived by the patient within four weeks and may extend over a long period.[3] (**A**) Alpha-blockers are effective in the treatment of BPH-LUTS, but their mechanism of action does not prevent disease progression, only relief of symptoms.[4,5] (**A**) The most common side effects of alpha-blockers are asthenia, dizziness and orthostatic hypotension.[5] (**A**) Ejaculatory dysfunction (retrograde ejaculation, reduction of seminal ejaculated volume) is frequently reported by patients.[6] (**A**)

5-alpha-reductase inhibitors (i5ARs)

There are currently two drugs that act by inhibiting 5AR: finasteride and dutasteride. Finasteride is a selective inhibitor of type II isoenzyme, whereas dutasteride is a non-selective inhibitor, affecting type I and II isoenzymes. i5ARs may be prescribed to men with lower urinary tract symptoms and enlarged prostate (> 40 mL) or high prostate-specific antigen (PSA) levels (> 1.6 ng/mL) and may prevent disease progression, reducing both the need for surgery and acute urinary retention.[4,7-10] (**A**)[11](**B**) One limitation to the use of monotherapy with i5ARs is the onset of action: improvement of BPH-LUTS takes between 4 and 6 months of therapy. Finasteride and dutasteride have similar efficacy in reducing prostate volume and improving urinary symptoms in BPH.[12] (**B**) The most relevant adverse effects include decreased libido, erectile dysfunction and ejaculation disorders. One to 2% of the patients develop gynecomastia (enlargement of the breasts with increased breast or nipple sensitivity).[13] (**A**)

Antimuscarinic drugs

These drugs have the property of inhibiting the action of acetylcholine and thus reducing the contractility of the detrusor muscle. Oxybutynin, tolterodine, darifenacin and solifenacin are currently available in Brazil. Muscarinic receptor antagonists should be considered in men with LUTS who have predominantly vesical storage symptoms.[14] (**A**) This class of drugs should be used with caution in men with BPH and infravesical obstruction, especially with high post-voidal residual volume, due to the possibility of incomplete emptying and the development of acute urinary retention.[14] (**A**) Common side effects include dry mouth, constipation, urinary difficulties, nasopharyngitis, dizziness, confusion and restlessness.

Beta-3 agonists

Beta-3 agonists, a new class of drugs for treatment of bladder storage symptoms, stimulates the sympathetic system during bladder filling, promoting relaxation of the detrusor muscle, increasing bladder functional capacity, decreasing urinary frequency and urinary urgency episodes, as well as urinary urge. Since it does not affect the parasympathetic system, there is no interference in detrusor contraction and, thus, in the voiding process, which minimizes the risk of urinary retention. Mirabegron, a beta-3 agonist, did not adversely affect voiding urodynamics (maximum urinary flow and detrusor pressure at maximum urinary flow) compared with placebo after 12 weeks of treatment in a double blind, placebo controlled study.[15] **(A)**

Combination therapy (antimuscarinic drugs and alpha-1 blocker)

Combination of anticholinergic drugs and alpha-1 blocker may reduce storage symptoms and urinary frequency, according to a long term study.[19] Although it can increase the risk of acute urinary retention in patients with BPH with baseline postvoid volume > 150 mL, the rate of acute urinary retention is reduced in patients with postvoid volume < 150 mL, around 1%.[16-19] (**A**)

Combination therapy (alpha-blocker and i5AR)

The combination of alpha-blockers and i5ARs is an effective treatment for patients with moderate to severe LUTS, increased prostate volume (> 40 mL), high PSA (> 1.6 ng/mL), and reduced maximum urinary flow. Combination therapy is valid not only for relief of symptoms but also to reduce the risk of progression of BPH, it means increase of the symptom score, surgical treatment due urinary retention, urinary incontinence, urinary tract infection and renal failure.[4,7-10] (**A**)[11](**B**) It is not recommended for treatment lasting less than one year and should be prescribed with

caution in men with suspected infravesical obstruction and high post-voidal residual urine volume.[7-11] (**A**)[12] (**B**). A multicenter, randomized, non-blinded study, that included treatment-naive patients with moderately symptomatic BPH, concluded that a fixed dose of dutasteride/tamsulosin may reduce the risk of clinical progression and improve symptoms compared to expectant treatment, with optional tamsulosin prescription (initiation of protocol-defined therapy if symptoms did not improve).[20] (**A**)

Phosphodiesterase-5 inhibitors (iPDE5s)

Several studies demonstrate the effect of iPDE5s on the treatment of BPH.[21-24] (**A**) The likely mechanisms of action stem from effects on smooth muscle relaxation, endothelial cell proliferation, improved blood flow, and activity on the prostatic efferent nerves.[25] (**D**) Currently, tadalafil (5 mg once daily) is approved in Brazil for the treatment of urinary symptoms associated with BPH. This indication is based on double-blind randomized placebo-controlled trials (RCT), that showed a significant reduction of I-PSS after twelve weeks.[22,23,26,27] (**A**) A prospective placebo-controlled study of tadalafil with tamsulosin used as an active comparator showed that tadalafil led to a decrease in tamsulosin-like I-PSS as early as week 1.[23] (**A**) Since iPDE5 is indicated for the treatment of erectile dysfunction, this class of medication may be an option in the treatment of patients presenting both disorders.[22,23,26] (**A**)

Combination therapy (iPDE5s and alpha-blockers)

The simultaneous use of iPDE5s and alpha-blockers may cause symptomatic hypotension. Such a risk can be reduced with tadalafil, uroselective alpha-blockers (tamsulosin, alfuzosin), low doses of alpha-blockers, separating doses for several hours instead of using them simultaneously, or waiting until the patient is taking a stable dose of a drug to initiate the second one.[28] (**D**) A systematic review with meta-analysis included seven RCTs [N=515 patients] and evaluated the efficacy of iPDE5s alone or combined with alpha-blockers in the treatment of erectile dysfunction and LUTS, with a follow-up ranging from 60 days to three months. For the treatment of erectile dysfunction, combination therapy produced a statistically significant increase in the International Index of Erectile Function-Erectile Function (IIEF-EF) compared with the group that used iPDE5s alone (MD 2.25, 95CI 0.07-4.43; six trials; I^2=78%). In the treatment of LUTS, the combination of iPDE5s and alpha-blockers produced statistically significant reductions in I-PSS score (MD -4,21, 95CI -7.09–-1.32; five trials; I^2=93%) and increase in maximum urinary flow (Q_{max}) [MD 1.43, 95CI 0.38-2.47; four trials; I^2=0%]. These results should be examined with caution on account of the high heterogeneity between studies for some outcomes, but we can infer that adding iPDE5s to alpha-blockers can improve BPH-LUTS.[29] (**A**)

Combination therapy (iPDE5s and i5ARs)

In an RCT with confidence intervals including clinically unimportant differences, 696 men aged ≥ 45 years old and with BPH were randomized to tadalafil 5 mg once daily plus finasteride 5 mg/day or placebo plus finasteride 5 mg/day for 26 weeks. Prostate symptoms were assessed by I-PSS. At baseline, all patients had I-PSS ≥ 13 points, prostate volume ≥ 30 cc and had never been treated with 5-phosphodiesterase inhibitors. 592 patients (85%) completed the study. Combination therapy (tadalafil + finasteride) compared to finasteride alone reduced the mean I-PSS after 26 weeks by 5.5 points vs. 4.5 points, respectively (minimum mean square error of 1 point, 95CI 0.2-1.9 points). For this comparison, the treatment-related adverse event rate (mild/moderate) was 31% vs. 27% (p-value was not reported). Combination therapy, compared to finasteride alone, showed patient improvement, but not an overall impression of clinical improvement.[30] (**A**) Therefore, the addition of tadalafil to finasteride may moderately improve urinary symptoms in men with BPH.[30] (**A**)

The addition of tadalafil to finasteride cannot increase the response to treatment clinically significantly at week 26, based on a post hoc secondary analysis of the RCT described above.[30] **(A)** Clinically significant response to treatment was defined as a reduction ≥ 3 points or ≥ 25% in the total I-PSS. Comparing the combination therapy (tadalafil + finasteride) with finasteride alone at week 26, the ≥ 3-point reduction in I-PSS was 71.4% vs. 70.2%, respectively (not significant) and the ≥ 25% reduction in I-PSS was 62% vs. 58.3% (not significant). Combination therapy was significantly associated with greater satisfaction with the treatment, in a pre-specified analysis.[31] (**B**)

In a subgroup analysis, also part of this study, including 404 sexually active men, tadalafil plus finasteride was associated with a higher score for erectile function compared to finasteride alone (p<0.001 for all moments analyzed).[30] (**A**)

Recommendation

- Men with mild or moderate symptoms, with minimal impact on quality of life, can be followed up with active monitoring. (**A**)
- Alpha-blockers are recommended in the treatment of BPH-LUTS, with rapid symptom improvement, but its mechanism of action does not interfere with disease progression. (**A**)

- i5ARs are recommended for men with LUTS, enlarged prostate, and/or high PSA levels. Results begin to be felt after a few months (4 to 6 months), but can prevent disease progression by reducing the need for surgery and acute urinary retention. (**A**)
- i5AR therapy combined with alpha-blockers is recommended for men with moderate to severe LUTS, enlarged prostate and/or high PSA levels, and reduced maximum urinary flow. (**A**)
- i5ARs therapy combined with alpha-blockers is not recommended for treatment lasting less than one year. (**A**)
- Muscarinic receptor antagonists and beta-3 agonists should be considered with caution in men with LUTS who have predominantly bladder storage symptoms. (**A**)
- Anticholinergic therapy, used alone or in combination with alpha-1 blocker, increases the risk of acute urinary retention in patients with BPH. (**A**)
- iPDE5s are recommended for the treatment of BPH--LUTS. This therapeutic class may be an option in the treatment of patients with erectile dysfunction and BPH-LUTS. (**A**)
- Addition of iPDE5s to alpha-blockers may improve BPH-LUTS. (**A**)
- Tadalafil plus finasteride is associated with an improvement in erectile function compared to finasteride alone. (**A**)
- Patients should be advised of the possible side effects of BPH drug therapy. (**D**)
- Due to the lack of scientific evidence, there is no recommendation for the use of herbal medicines in the treatment of BPH-LUTS. (**A**)

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Therapeutic use of the rebound effect of modern drugs: "New homeopathic medicines"

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Article received: 7/4/2016
Accepted for publication: 7/9/2016

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http://dx.doi.org/10.1590/1806-9282.63.02.100

Summary

The homeopathic treatment is based on the principle of therapeutic similitude, employing medicines that cause certain disorders to treat similar manifestations, stimulating a reaction of the organism against its own ailments. The occurrence of this secondary reaction of the organism, opposite in nature to the primary action of the medicines, is evidenced in the study of the rebound (paradoxical) effect of several classes of modern drugs. In this work, in addition to substantiate the principle of similitude before the experimental and clinical pharmacology, we suggest a proposal to employ hundreds of conventional drugs according to homeopathic method, applying the therapeutic similitude between the adverse events of medicines and the clinical manifestations of patients. Describing existing lines of research and a specific method for the therapeutic use of the rebound effect of modern drugs (http://www.newhomeopathicmedicines.com), we hope to minimize prejudices related to the homeopathy and contribute to a broadening of the healing art.

Keywords: homeopathy, pharmacology, pharmacodynamic action of homeopathic remedy, law of similars, rebound effect, new homeopathic remedy.

Introduction

The homeopathic model for the treatment of disease is based on four assumptions: (i) the principle of healing by similars; (ii) pathogenetic experimentation of medicines in health humans; (iii) use of ultra-diluted (dynamized) medicines; and (iv) prescription of individualized medicines. Although great importance is attributed to dynamized medication (produced through the dilution and serial agitations of the substances), incorporated secondarily to the therapy in order to minimize possible initial symptomatic aggravations derived from the application of the principle of healing by similars, the first two assumptions are the foundation of the homeopathic episteme, with individualized homeopathic medicine (chosen according to the totality of characteristic signs and symptoms) holding the inherent condition for awakening the organism's healing reaction.[1]

In Ancient Greece, Hippocrates taught that diseases could be treated by the principles of "contraries" *(contraria contrariis curantur)* or "similars" *(similia similibus curantur)*, recommendations which were followed by several exponents of subsequent medical schools.[2]

At present, the "principle of contraries" is applied to a large part of conventional therapy, which uses medicines with primary action against (anti-) the signs and symptoms of diseases (palliative or antipathic drugs) in order to minimize or neutralize their manifestations. On the other hand, the "principle of similars" is used by homeopathic therapy, which uses medicines that cause similar signs and symptoms (homeo) to diseases in order to stimulate a secondary action or reaction by the organism against its own disorders.

Since 1998, we have been scientifically grounding the principle of therapeutic similarity through the systematic study of the "rebound effect" of modern drugs ("paradoxical reaction" of the organism),[2-12] showing the manifestation of this secondary and opposite reaction of the organism after the primary action of numerous classes of drugs. At the end of 2013, we published a review on the rebound effect of drugs in this journal,[13] showing the extent of the phenomenon and alerting health professionals about the serious consequences that this unknown adverse event can cause.

In the last decade, exponents of modern pharmacology have suggested a therapeutic strategy entitled "para-

doxical pharmacology," similar to the one propagated by homeopathy for over two centuries, proposing the use of conventional drugs that cause an exacerbation of the disease in the short term in order to treat the same disease in the long term.[14-26] Similarly, since the beginning of our studies, we have been suggesting the use of modern drugs in accordance with the principle of therapeutic similarity, proposing the use of drugs that cause adverse events similar to the manifestations of diseases in order to treat them homeopathically, using the rebound effect (paradoxical reaction) in a curative manner. [2,3,9,10,11,13,27-33]

In this study, we elaborate on this proposal in accordance with the assumptions of the homeopathic model and the fundamentals of modern pharmacology, describing existing research and a specific methodology for the therapeutic application of the rebound effect of modern drugs, suggesting the use of this practice in a complementary and adjuvant manner[34] in a myriad of diseases and syndromes. As such, we hope to contribute to medical knowledge, minimizing prejudice related to homeopathy and encouraging an expansion of the art of healing.

The principle of similarity according to the homeopathic model

In the development of the homeopathic model, Samuel Hahnemann (1755-1843) used the phenomenological method of qualitative research to describe the effects of drugs on human physiology and substantiate the principle of similarity. Based on the study of the pharmacological properties of dozens of medicinal substances of his time, in which a "secondary action or reaction of the organism after primary drug action" was observed,[35] Hahnemann enunciated an aphorism to explain the possible effects of drugs on human health:

> Every agent that acts upon the vitality, every medicine, deranges more or less the vital force, and causes a certain alteration in the health of the individual for a longer or a shorter period. This is termed *primary action*. [...] To its action our vital force endeavors to oppose its own energy. This resistant action is a property, is indeed an automatic action of our life-preserving power, which goes by the name of *secondary action* or *counteraction*. (*Organon of medicine*, § 63)[36]

He exemplifies this principle describing the primary actions of medicines on various physiological systems and the consequent secondary actions or counteractions of the organism, with opposite effects to the primary physiological changes, which induce the organism to return to the state prior to the intervention (conservation force or vital reaction or life-preserving power):

> (...) Excessive vivacity follows the use of strong coffee (primary action), but sluggishness and drowsiness remain for a long time afterwards (reaction, secondary action), if this be not always again removed for a short time by imbibing fresh supplies of coffee (palliative, short duration). After the profound stupefied sleep caused by opium (primary action), the following night will be all the more sleepless (reaction, secondary action). After the constipation produced by opium (primary action), diarrhea ensues (secondary action); and after purgation with medicines that irritate the bowels, constipation of several days' duration ensues (secondary action). And in like manner it always happens, after the primary action of a medicine that produces in large doses a great change in the health of a healthy person, that its exact opposite, when, as has been observed, there is actually such a thing, is produced in the secondary action by our vital force. (*Organon of medicine,* § 65)[36]

In order to provide clarification to readers not familiar with the homeopathic terminology, the primary actions (direct effects) of the medicines correspond to the therapeutic and adverse effects of modern pharmacology, and the secondary actions (indirect effects) of the medicines or reactions of the organism correspond to the rebound effect of drugs or paradoxical reaction of the organism, which we will describe in detail below. Similarly, the terms conservation force or vital reaction or life-preserving power correspond to the homeostatic mechanisms described by modern physiology, that is, the property of living organisms to maintain the constancy of the internal environment through automatic self-adjustments to physiological processes, ranging from simple cellular mechanisms to complex psychological functions.

Whereas this reaction of the organism (secondary and opposite reaction to the primary action of the medicine) may manifest with all types of drugs, regardless of the dose, and in any individual, Hahnemann raises the principle of similarity to the category of "natural phenomenon" (*Organon of medicine,* § 58, 61, 110-112).[36]

Proposing to administer substances to patients which arouse similar symptoms in individuals submitted to "homeopathic pathogenetic experimentation" ("pathogenetic homeopathic trials," similar to phase I pharmacological clinical trials),[37,38] the principle of therapeutic similarity aims to stimulate a curative homeostatic reaction, inducing the organism to react against its own disorders.

The principle of similarity in the light of modern pharmacology[2-13]

Building a bridge between the principle of similarity and modern scientific rationality, hundreds of studies de-

scribed, in recent medical literature, the occurrence of secondary and opposite reactions of the organism after the primary actions of different drugs, confirming the homeopathic postulate. As mentioned previously, this secondary action or organism's reaction, which manifests itself automatically and instinctively in order to maintain the homeostasis of the system, is described by contemporary pharmacology and physiology as the rebound effect of the drugs, or paradoxical reaction of the organism, respectively. Analogously, the primary action of the drugs cited by Hahnemann corresponds to the therapeutic, adverse and collateral effects of modern drugs.

According to evidence from experimental and clinical pharmacology,[2-13] the rebound effect of modern drugs presents similar characteristics to the secondary action or the reaction of the organism described by the homeopathic model (*Organon of medicine,* § 59, 64, 69):[36] (i) it causes an opposite reaction of the organism with greater intensity than the primary action of the drug; (ii) it occurs after the primary action of the drug as an automatic manifestation of the organism; (iii) it is independent of the drug, dose, duration of treatment or the type of symptom (illness); (iv) its magnitude is proportional to the primary action of the drug; and (v) it only manifests in susceptible individuals (idiosyncratic character).

According to the extensive literature,[2-13] several studies illustrate the universality of the rebound phenomenon in relation to the distinct classes of palliative drugs (antianginal, antihypertensive, antiarrhythmic, antithrombotic, and antihyperlipidemic agents, anxiolytics, sedative-hypnotics, neurostimulants, antidepressants, antipsychotics, anti-inflammatory drugs, analgesics, diuretics, bronchodilators, anti-dyspeptics, bone antiresorptive agents, and immunomodulators, among others), showing aggravation of the signs and symptoms initially suppressed by the primary and direct action of the drug, after its discontinuation.

By definition, the rebound effect presents an intensity and/or frequency a few times higher than the corresponding baseline symptoms suppressed by the primary action of the antipathic drug, a characteristic that distinguishes the rebound phenomenon from the natural reappearance of chronic symptoms after the end of treatment. Epidemiological studies show that this magnitude can cause severe and fatal events after the suspension of certain classes of drugs (antithrombotics, antidepressants, bronchodilators, anti-dyspeptics and immunomodulators, among others).[4-13]

The rebound effect manifests itself at different intervals (hours to weeks) after the exhaustion of the biological effect (half-life) of the drug, and its duration is also variable. Despite the suspension or discontinuation of the drug being a prerequisite for the manifestation of the rebound effect, given that the primary action of the drug persists while the receptors are being stimulated (biological half-life), studies show that the rebound effect may also occur in the course of treatment due to therapeutic failure or the development of tolerance, tachyphylaxis or desensitization. On the other hand, a slow and gradual reduction of doses (tapering) to prevent abrupt discontinuation can minimize the occurrence of the rebound effect.[2-13]

In analogy to the proposal to be detailed below, reports in the literature describe the use of conventional drugs according to therapeutic similarity. Among these, we can cite the use of biphasic oral contraceptives to promote ovulation and rebound pregnancy in women with functional sterility and the use of central nervous system stimulants (methylphenidate) to treat attention deficit hyperactivity disorder (ADHD).[39,40]

Paradoxical pharmacology[14-26]

A strategy suggested by Richard A. Bond in 2001,[14] paradoxical pharmacology proposes the therapeutic use of the paradoxical effects of drugs (secondary reactions of the organism with the opposite nature to the primary effects of the drugs). Universal in nature, such paradoxical, bidirectional or compensatory effects appear in several classes of drugs, regardless of the dose, and affect various percentages of individuals. Although not fully elucidated, this paradoxical effect is manifested at different levels of the biological self-regulation systems, increasing the functional complexity of the entire organism, from subcellular components (channels, enzymes, receptors, transporters, organelles, etc.) to cells, tissues and organs.[15-19]

Present in any physiological system, these paradoxical and bidirectional effects occur due to varying mechanisms: different actions on the same receptor, due to temporal effects (for example, beta-blockers with intrinsic sympathomimetic activity); stereochemical effects (for example, salbutamol); multiple receptor targets, with or without associated temporal effects (for example, procainamide); antibody-mediated reactions (for example, heparin-induced thromboembolism); pharmacokinetic effects of competing compartments (for example, bicarbonate); interruption and non-linear effects on systems (for example, dopaminergic agents); systemic overcompensation (for example, antiretroviral therapy and immune reconstitution inflammatory syndrome); other higher level feedback mechanisms (for example, digoxin) and multilevel feedback cycles (for example, isotretinoin-associated acne fulminant), and more.[19]

As described for the rebound effect,[2-13] the authors cite several examples of paradoxical and bidirectional effects of drugs in different pharmaceutical classes and physiological systems: immunomodulators (systemic corticoids and TNF-α inhibitors), anticancer drugs (chemotherapy, radiotherapy and arsenic), antiarrhythmics (procainamide and isoproterenol), antihypertensives (methyldopa, clonidine, guanabenz, moxonidine and thiazides), vasodilators (nitrates), drugs for heart failure (beta-blockers, ACE inhibitors, angiotensin II receptor blockers and hydralazine), lipid modifying drugs (fibrates and ezetimibe), inotropic and chronotropic drugs (isoproterenol, epinephrine, beta-blockers and calcium channel blockers), vasoconstrictors (ergot alkaloids and vasopressin), anesthetics (sevoflurane, ketamine and propofol), antiepileptic drugs (benzodiazepines, barbiturates and hydantoin), sedative-hypnotics (anticholinergics, antihistamines, antispasmodics, barbiturates, benzodiazepines, bromides, chloral hydrate, ethanol and opioids), psychotropic drugs (antidepressants and antipsychotics), peripheral nervous system drugs (acetylcholinesterase and capsaicin inhibitors), antidyskinetic drugs (dopaminergic agents), acid-base agents (sodium lactate and bicarbonate), bone metabolism agents (parathyroid hormone and bisphosphonates), electrolytes (hypertonic saline and magnesium hydroxide), glycemic agents (insulin and hypoglycemic agents), steroid hormones (dexamethasone), thyroid agents (iodine and lithium), antihyperuricemic agents (xanthine oxidase and urate oxidase inhibitors), gastrointestinal agents (opiates, cholecystokinin and ceruletide), hematological agents (erythropoietin, vitamin K antagonists and adenosine diphosphate receptor inhibitors), bronchodilators (short- and long-acting beta-adrenergic bronchodilators), dermatological agents (histamine receptor inhibitors, high-intensity long-wave ultraviolet light and 8-methoxypsoralen), and more.[19]

For Bond,[14] a possible hypothesis to explain the functioning of paradoxical pharmacology is the "difference between acute and chronic effects of drugs." Reiterating that the acute and chronic responses to drugs may differ substantially, often being of opposite natures, he proposes that "the exacerbation of a disease can make the compensatory and redundant mechanisms of the organism achieve a beneficial long-term response." This is particularly evident in events mediated by receptors: acute exposure to inhibitors can produce receptor activation and increased signaling, while chronic exposure can produce receptor desensitization and decreased signaling. The same phenomenon occurs with receptor inhibitors.

Similar to the homeopathic method of treatment, which uses ultra-diluted doses of medicines with the aim of avoiding possible aggravation of the illness after application of therapeutic similarity, as a general rule, proponents of paradoxical pharmacology suggest starting with "very small doses, increasing them gradually over the following weeks."[14]

Exemplifying the therapeutic use of the paradoxical reactions of the organism, the authors describe clinical conditions which can be treated using this proposal. Congestive heart failure (CHF) is a disease related to impaired cardiac contractility, in which the acute use of beta-adrenergic receptor inhibitors increases cardiac contractility, improves hemodynamics and reduces related symptoms. However, chronic use results in increased mortality. On the other hand, while the short-term use of beta-adrenergic antagonists (beta-blockers: carvedilol, metoprolol, bisoprolol, among others) decreases contractility and exacerbates the CHF, causing the worsening of the illness, long-term use results in increased cardiac contractility and decreased mortality.[14,18-20] The same is observed with calcium channel blockers.[21]

Similarly, beta-adrenergic agonists are the most potent bronchodilators and play an important role in all stages of asthma management. However, as mentioned in the study of the rebound effect, chronic use is associated with irreversible and fatal paradoxical bronchospasms. On the other hand, while short-term use of beta-adrenergic antagonists leads to bronchoconstriction and worsening of asthma, long-term use leads to bronchodilation and increased asthma management.[14,18,22,23]

Additional examples include the use of methylphenidate (a central nervous system stimulant) in the treatment of ADHD and the use of 5-HT1A serotonin receptor agonists (mediators of hyperalgesia) to produce analgesia.[18] Of ancient knowledge, the use of thiazide class diuretics provides a paradoxical antidiuretic benefit in the treatment of diabetes insipidus, reducing polyuria and increasing urine osmolality.[24]

Arsenic trioxide (As_2O_3), an important carcinogen, has been used by homeopathy for more than two centuries as an adjuvant drug in the treatment of several types of cancer, and is being used by paradoxical pharmacology as a promising anticancer drug,[25,26] mainly in the relapse of acute promyelocytic leukemia,[41,42] including in Brazil,[43,44] among other applications.[19]

Therapeutic use of the rebound effect of modern drugs: "new homeopathic medicines"[27-33]

Reiterating that homeopathic treatment has the prerogative of using drugs that cause pathogenetic manifes-

tations (signs, symptoms, physiological or pathological changes, etc.) similar to disorders requiring treatment, it can be applied with any substance (natural or synthetic) and at any dose (by massive or infinitesimal), provided this principle of similarity is observed. Thus, conventional drugs can be employed according to the homeopathic premises provided they cause primary effects (therapeutic, adverse or collateral effects) similar to the totality of individual characteristic manifestations.

In this proposal,[27-33] we are suggesting the use of the rebound effect of modern drugs in a curative manner, administering ultra-diluted doses (dynamized medicines) to patients of the drugs that cause a set of similar adverse events in phases I-IV pharmacological clinical trials, proposing to stimulate a homeostatic response of the organism against its own disorders.

To make this project possible, a *Homeopathic Materia Medica of Modern Drugs*[29] was elaborated, systematizing all of the primary or pathogenic effects (therapeutic, adverse and collateral effects) to 1,250 modern drugs described in *The United States Pharmacopeia Dispensing Information* (USPDI),[45] according to an anatomical and functional distribution (systems or tracts) and in accordance with the dynamics used in the chapters of the classic *Homeopathic Materia Medica*.[46]

In order to facilitate the selection of the individualized medicines according to the totality of manifestations similar to the patient-disease binomial, an essential premise for the success of homeopathic treatment, the second stage of the project involved the elaboration of a *Homeopathic Repertory of Modern Drugs*,[29] where the pathogenetic effects and their corresponding medicines are organized in the same anatomical/functional distribution, following the arrangement of the classic homeopathic repertories.[47]

Titled *New Homeopathic Medicines: Use of Modern Drugs According to the Principle of Similitude*,[29] this project is described and systematized in three digital compendia *(Scientific Basis of the Principle of Similitude in Modern Pharmacology, Homeopathic Materia Medica of Modern Drugs* and *Homeopathic Repertory of Modern Drugs)* provided on a bilingual site with free access (http://www.newhomeopathicmedicines.com), allowing the proposal to be known and applied by all interested colleagues.

Exemplifying this possible 'off label' use of numerous classes of modern drugs according to the principle of therapeutic similarity, dozens of therapeutic drugs that show increased blood pressure as primary effect (adalimumab, cyclosporine, dopamine and anti-inflammatory drugs, among others) could be used homeopathically to treat hypertension, provided that other primary or pathogenetic effects of the drug present similarity with the set of signs and symptoms of the individual patient. Respecting this individualization of medicine, drugs that increase blood glucose (amprenavir, corticotropin, diazoxide and estrogens, and more) could be employed homeopathically to treat diabetes; drugs that cause inflammation of the gastric mucosa (abacavir, anti-inflammatory drugs, carbidopa and cilostazol, among others) could be employed homeopathically to treat gastritis and gastric ulcers; medicines that cause immunosuppression (cyclosporins, corticoids and immunosuppressants, and more) could be employed to stimulate the immune system of immunosuppressed patients; among others. Table 1 describes some examples of possible applications of therapeutic similarity with modern drugs in disorders, diseases and syndromes, in accordance with the adverse events caused by such on the various systems or tracts of individuals and described in phase I-IV pharmacological clinical trials.[29,45]

Use of dynamized estrogen in the treatment of chronic pelvic pain associated with endometriosis

Endometriosis is a chronic inflammatory disease characterized by the implantation and proliferation of endometrial tissue in extrauterine locations, causing chronic pelvic pain that is difficult to control. As the main pathophysiological aspect, it is worth mentioning that endometriosis is an estrogen-dependent disease. Putting into practice the proposal described above, we developed a clinical research protocol to assess the effect of dynamized estrogen in the treatment of endometriosis-associated pelvic pain (dysmenorrhea, deep dyspareunia, non-cyclic pelvic pain, cyclic bowel pain and/or cyclic urinary pain).

In the study of modern drugs according to the USPDI[45] and, consequently, in the *Homeopathic Repertory of Modern Drugs* (Chapter "Female Genitalia"),[29] we find a description of the specific pathological sign of endometriosis ("endometrial proliferation or hyperplasia") as an adverse event in four classes of conventional drugs (systemic and vaginal estrogens, tamoxifen and toremifene) (Table 2).

One of those drugs, "systemic estrogen," presents a set of adverse events (pathogenetic effects) that are quite similar to the main manifestations of endometriosis syndrome (endometrial proliferation, dysmenorrhea, dyspareunia, abdominal pain, depression, anxiety, insomnia and migraine, among others) (Table 3), and was selected for the study due to this particularity (individualization of the medicine).

In this randomized, double-blind and placebo controlled trial (RCT), 50 patients with endometriosis, chronic pelvic pain refractory to conventional hormone thera-

TABLE 1 Possible applications of therapeutic similitude with modern drugs.

Anatomical and functional distribution	Possible applications of therapeutic similitude (Adverse events or pathogenetic effects caused in individuals)
Mind/psyche	agitation, amnesia, anxiety, coma, delirium, dementia, depression, disorientation, memory lapses, hyperactivity, irritability, lethargy, mania, nervousness, panic, schizophrenia, suicidal disposition, and more
Head	cerebral aneurysm, stroke, encephalitis, intracranial hypertension, meningitis, headache/migraine, dizziness/vertigo, gait disorders, orthostatic hypotension, syncope, instability, and more
Eyes	astigmatism, atrophies, bleeding, cataract, chemosis, corneal diseases, dryness, glaucoma, inflammation, keratopathy, necrosis, neuritis, nystagmus, papilledema, paralysis, pupil disorders, retinal disorders, and more
Vision	amblyopia, blindness, diplopia, hyperopia, myopia, presbyopia, scotoma, and more
Hearing	tinnitus, deafness, hyperacusis, hearing loss, and more
Nose	congestion, coryza, dryness, epistaxis, rhinitis, sinusitis, sneezing, and more
Face	motor tics, heat waves, hirsutism, neuritis, paralysis, swelling, trismus, and more
Mouth	severe bleeding, discoloration, dryness, gingivitis, glossitis, mucositis, sialorrhea, speech disorders, stomatitis, taste disorders, ulcers, and more
Throat	angioedema, dryness, dysphagia, esophagitis, pharyngitis, ulcers, and more
Outer throat	goiter, heat waves, thyroid disorders, lymphadenopathy, parotitis, pain/stiff neck, and more
Stomach	anorexia, dyspepsia, eructation, gastritis, gastroenteritis, hemorrhage, hiccups, nausea, polydipsia, reflux, ulcer, vomiting, and more
Abdomen	ascites, appendicitis, cholecystitis, cholestasis, cirrhosis, colitis, gastroenteritis, hemorrhage, liver disorders (failure, necrosis, steatosis, hepatitis, hepatomegaly), paralytic ileus, inflammatory bowel disease, intestinal obstruction or perforation, malabsorption syndrome, pancreatitis, peritonitis, splenomegaly, tumors, and more
Rectum	constipation, diarrhea, hemorrhage, hemorrhoid, mucositis, tenesmus, and more
Bladder	hemorrhage, inflammation, urination disorders, and more
Kidneys	calculi, edema, inflammation (interstitial, glomerulonephritis, pyelonephritis), renal failure, tubular disorders, and more
Urine	ketonuria, albuminuria, glycosuria, hematuria, proteinuria, pyuria, sediments, and more
Male genitalia	testicular atrophy, sexual desire disorders, edema, sexual dysfunction (ejaculation, erection, infertility, orgasm), inflammation, and more
Female genitalia	abortion, cancer, contraception, sexual desire disorders, sexual dysfunction, hemorrhage, hormonal dysfunction, endometriosis, inflammation, menstrual disorders, ovarian disorders, uterine disorders, tumors, and more
Larynx and trachea	inflammation, laryngism, edema (glottis, larynx), and more
Breathing	types (fast, trapped, interrupted, irregular, slow), asthma, bronchitis, dyspnea, respiratory failure, wheezing, rattles, and more
Chest/thorax	acute myocardial infarction, angina pectoris, arrhythmias (atrial fibrillation, heart block, ventricular tachycardia), heart failure, pericardial or pleural effusion, inflammation (alveolitis, endocarditis, pneumonitis, pericarditis, pleuritis), pulmonary disorders (edema, embolism, fibrosis), adult respiratory distress syndrome, and more
Extremities	arthrosis, ataxia, edema, exostosis, fracture, gout, incoordination, inflammation (arthritis, myositis, neuritis, phlebitis, tendinitis), myopathy, neuropathies, osteoporosis, paralysis, rigidity, weakness, and more
Skin	acne, allergies, alopecia, inflammation (cellulitis, dermatitis, eczema), necrosis, pemphigus, psoriasis, purpura, seborrhea, and more
General	anaphylaxis, anemia, anesthesia, seizures, demyelinating diseases, diabetes, edema, encephalopathy, fatigue, hypertension, hyperthermia, hypotension, hypothermia, influenza, lymphadenopathy, neuropathy, thromboembolism, weight (gain/loss), tumors or cancer, and more

TABLE 2 Endometriosis in the *Homeopathic Repertory of Modern Drugs* (Chapter "Female Genitalia")[29]

Endometrium
Endometriosis; disorder, endometrial; proliferation, endometrial; hyperplasia: DrosE-syst., Estro-syst., Estro-vag., Tamo-syst., Tore-syst.

DrosE-syst.: drospirenone and estradiol (systemic); Estro-syst.: estrogens (systemic); Estro-vag.: estrogens (vaginal); Tamo-syst.: tamoxifen (systemic); Tore-syst.: toremifene (systemic).

TABLE 3 Adverse events or pathogenetic effects of estrogen (systemic).

Anatomical and functional distribution	Adverse events or pathogenetic effects of estrogen (*The United States Pharmacopeia Dispensing Information*)[45] (*Homeopathic Materia Medica of Modern Drugs*)[29]
Mind/psyche	**anxiety; depression;** *emotional lability; dementia; irritability; nervousness*
Head	**headache; migraine; dizziness;** *embolic stroke; chorea; worsening of epilepsy; hair loss*
Eyes	*Herpes simplex; contact lens intolerance; increased corneal curvature (changes in vision); optic neuritis; retinal vein occlusion (thrombosis)*
Vision	*visual disturbances*
Nose	**sinusitis; nasopharyngitis; rhinitis;** *nasal congestion; herpes simplex*
Face	*acne; hirsutism; herpes simplex; neuritis; chorea*
Tongue	*chorea*
Teeth	*abscesses*
Throat	**nasopharyngitis**; **pharyngitis**
Outer throat	**neck pain**
Stomach	**anorexia; dyspepsia; nausea**; *gastroenteritis; vomit; changes in appetite*
Abdomen	**bloating, flatulence and abdominal cramps;** *gastroenteritis; biliary obstruction; pancreatitis; liver changes (hemangioma; enzymes)*
Rectum	**constipation**; *diarrhea*
Bladder	**urinary tract infection**
Kidneys	**urinary tract infection**
Female genitalia	**amenorrhea**; **dysmenorrhea**; **vaginitis**; **fungal vaginosis**; *metrorrhagia; dyspareunia; vaginal hemorrhage; candidiasis; herpes simplex; increased libido; menorrhagia; stains; endometrial atrophy; endometrial or ovarian cancer; cervical ectropion; endometrial hyperplasia; uterine leiomyoma; changes in cervical mucus*
Breathing	**bronchitis;** *asthma exacerbation*
Coughing	*increase in coughing*
Chest/thorax	**bronchitis; increased breast size, sensibility or pain; gynecomastia;** *pleural infection; chest pain; breast cancer; fibroadenomas; pulmonary embolism; galactorrhea; myocardial infarction; palpitation; arrhythmias; coronary artery diseases*
Back	**pain**
Extremities	**peripheral edema;** *cramps; muscle spasms; osteoarthritis; neuritis; chorea; worsening of varicose veins*
Sleep	**insomnia**
Skin	**irritation; pruritus; rashes; redness**; *acne; hirsutism; chloasma; hemorrhagic rash; erythema multiforme; erythema nodosum; melasma*
General	**asthenia; flu syndrome; hypersensitivity reactions; infections; pain; weight gain;** *fatigue; hepatitis; hypertension; hypoesthesia; thromboembolism; hypocalcemia; cholestatic jaundice; worsening of systemic lupus erythematosus; worsening of porphyria; sodium retention; diabetes or reduced glucose tolerance; thrombophlebitis; increased triglycerides*

Markdown text (font/letter style) to describe adverse events corresponds to their incidence frequency (%) within the population (USPDI, phase I-IV clinical trials): score 4/**bold** (≥ 4%); score 3/*italic and underlined* (1-4%); score 2/*italic* (< 1%); score 1 / normal (overdose).

py and a set of signs and symptoms similar to the adverse events or pathogenetic effects of estrogen (Table 3) were recruited at the Endometriosis Sector of the Clinical Gynecology Division of the University of São Paulo Medical School's Hospital das Clínicas (HC-FMUSP). The selection process was carried out through an analysis of patient records and responses to structured questionnaires. After meeting the inclusion criteria, the patients were distributed randomly to receive dynamized estrogen or placebo, while maintaining conventional hormone therapy. The primary clinical outcome was the severity of the chronic pelvic pain after 24 weeks of intervention.[48,49] The results of this recently published RCT showed that dynamized estrogen was significantly more effective than placebo for reducing endometriosis-associated pelvic pain (dysmenorrhea, non-cyclic pelvic pain and cyclic bowel pain), improving quality of life (bodily pain, vitality and mental health) and reducing depressive symptoms.[50]

In a similar proposal described in another recently published paper[51] that demonstrated worsening of psoriasis (rebound psoriasis) after discontinuation of immunomodulatory drugs (T-cell modulators and TNF inhibitors), we suggest the treatment of psoriasis with drugs that cause psoriasis as an adverse event (beta-ad-

renergic blocking agents, lithium and antimalarial agents, among others) corresponding with the use of dynamized estrogen to treat endometriosis.

Conclusion

Describing the undesirable effects of the indiscriminate use of drugs that act according to the principle of contraries, opposite to the principle of similars, Hahnemann warned of the risks of secondary action (rebound effect or paradoxical reaction) of the organism, validating the principle of similarity through the Aristotelian syllogism (*modus tollens,* denying the consequent or indirect proof):

> If these ill-effects are produced, as may very naturally be expected from the antipathetic employment of medicines, the ordinary physician imagines he can get over the difficulty by giving, at each renewed aggravation, a stronger dose of the remedy, whereby an equally transient suppression is effected; and as there then is a still greater necessity for giving ever-increasing quantities of the palliative there ensues either another more serious disease or frequently even danger to life and death itself, but never a cure of a disease of considerable or of long standing. (*Organon of medicine,* § 60)[36]

With modern drugs, a large number of iatrogenic events could be avoided if health professionals paid attention to the possible occurrence of the rebound effect or paradoxical reaction of the organism,[13] minimizing the aggravation of clinical conditions with the slow and gradual decrease of doses. Although not included in conventional adverse events of drug, "effects from the discontinuation of drugs are part of the pharmacology of the drug"[52] and should be incorporated into the teaching of modern pharmacology.

On the other hand, by employing the rebound effect of conventional drugs in a curative manner, we can expand the spectrum of therapeutic similarity with hundreds of "new homeopathic medicines," including signs and symptoms that are absent in classic homeopathic pathogenetic trials and allowing the application of homeopathic treatment for a multitude of diseases, disorders and syndromes.

As has been suggested by the propagators of homeopathic therapy for more than two centuries,[28,33] Bond and Giles[18] encourage researchers to examine the paradoxical phenomenon (rebound effect) without prejudice and to challenge the dogma of current treatment paradigms with new therapeutic approaches, despite the difficulty in accepting new ideas by our peers.

Resumo

Uso terapêutico do efeito rebote dos fármacos modernos: "Novos medicamentos homeopáticos"

O tratamento homeopático está fundamentado no princípio da similitude terapêutica, empregando medicamentos que causam determinados distúrbios para tratar manifestações semelhantes, estimulando uma reação do organismo contra seus próprios transtornos. A ocorrência dessa reação secundária do organismo, de natureza oposta à ação primária dos medicamentos, está evidenciada no estudo do efeito rebote (paradoxal) de inúmeras classes de fármacos modernos. Neste trabalho, além de fundamentar o princípio da similitude perante a farmacologia clínica e experimental, sugerimos uma proposta para empregar centenas de drogas convencionais segundo o método homeopático, aplicando a similitude terapêutica entre os eventos adversos dos medicamentos e as manifestações clínicas dos pacientes. Descrevendo linhas de pesquisa existentes e um método específico para o uso terapêutico do efeito rebote dos fármacos modernos (http://www.novosmedicamentoshomeopaticos.com), esperamos minimizar preconceitos relativos à homeopatia e contribuir para uma ampliação da arte de curar.

Palavras-chave: homeopatia, farmacologia, ação farmacodinâmica do medicamento homeopático, lei dos semelhantes, efeito rebote, medicamentos homeopáticos novos.

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Targeting personalized medicine in a non-Hodgkin lymphoma patient with ^{18}F-FDG and ^{18}F-choline PET/CT

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Article received: 5/18/2016
Accepted for publication: 5/19/2016

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http://dx.doi.org/10.1590/1806-9282.63.02.109

Summary

Early diagnosis and staging of non-Hodgkin lymphoma (NHL) is essential for therapeutic strategy decision. Positron emission tomography/computed tomography (PET/CT) with fluordeoxyglucose (FDG), a glucose analogue, labeled with fluor-18 (^{18}F-FDG) has been used to evaluate staging, therapy response and prognosis in NHL patients. However, in some cases, ^{18}F-FDG has shown false--positive uptake due to inflammatory reaction after chemo and/or radiation therapy. In this case report, we present a NHL patient evaluated with ^{18}F-FDG and ^{18}F-choline PET/CT scan imaging pre- and post-therapy. ^{18}F-FDG and ^{18}F-choline PET/CT were performed for the purpose of tumor staging and have shown intense uptake in infiltrative tissue as well as in the lymph node, but with some mismatching in the tumor. Post-treatment ^{18}F-FDG and ^{18}F-choline PET/CT scans revealed no signs of radiotracer uptake, suggesting complete remission of the tumor. ^{18}F-choline may be a complimentary tool for staging and assessment of therapeutic response in non-Hodgkin lymphoma, while non-^{18}F-FDG tracer can be used for targeted therapy and patient management.

Keywords: ^{18}F-FDG, ^{18}F-choline, PET/CT, non-Hodgkin lymphoma, neoplasm staging.

Early diagnosis and staging of non-Hodgkin lymphoma (NHL) is an essential process that involves many different technologies.[1] Hybrid positron emission tomography/computed tomography (PET/CT) with fluordeoxyglucose (FDG), a glucose analogue, labeled with fluor-18 (^{18}F-FDG) has been largely used to evaluate staging, therapy response and prognosis in NHL patients.[2,3] PET/CT combines morphological and metabolic information of the cancerous tissue, providing more accurate data regarding its different behaviors.[2,3,4] However, in some cases, ^{18}F-FDG has shown false-positive uptake due to inflammatory reaction after chemo and/or radiation therapy.[5,6] In order to overcome this problem and increase the accuracy of malignant cell detection, especially to assess response to different therapeutic modalities, non-^{18}F-FDG PET radiotracers might be an interesting strategy. Thus, in this study, we present the case of a patient diagnosed with NHL assessed through PET/CT scan imaging using ^{18}F-FDG and ^{18}F-choline pre- and post-chemoradiation therapy, in order to determine and improve specific patient management.

A 61-year-old male patient, in good general condition at physical examination, presented an irregular ulcerated lesion in the anterior chest wall. ^{18}F-FDG PET/CT imaging, performed for staging, showed intense uptake in infiltrative tissue into pectoral muscles reaching the sternal body with involvement of supra- and infradiaphragmatic lymph nodes (Figure 1A and E). Further pre-therapy, ^{18}F-choline PET/CT scans were consistent with increased radiotracer uptake in the lesion site as well as in the lymph nodes (Figure 1B and F), but with some mismatching in the tumor (Figure 1F). Histological and immunohistochemistry examinations revealed areas of dense and diffuse infiltrate of large anaplastic cells with strong positivity for CD20 and CD10, and a high proliferative profile with strong and diffuse positivity for Ki-67, that corroborate with the diagnosis of cutaneous large-B cells non-Hodgkin lymphoma (NHL) (Figure 1G and H). The patient was then referred for oncological treatment, thus receiving eight cycles of lymphoma standard (R-chop) chemotherapy followed by conformational radiotherapy (30,6 Gy)

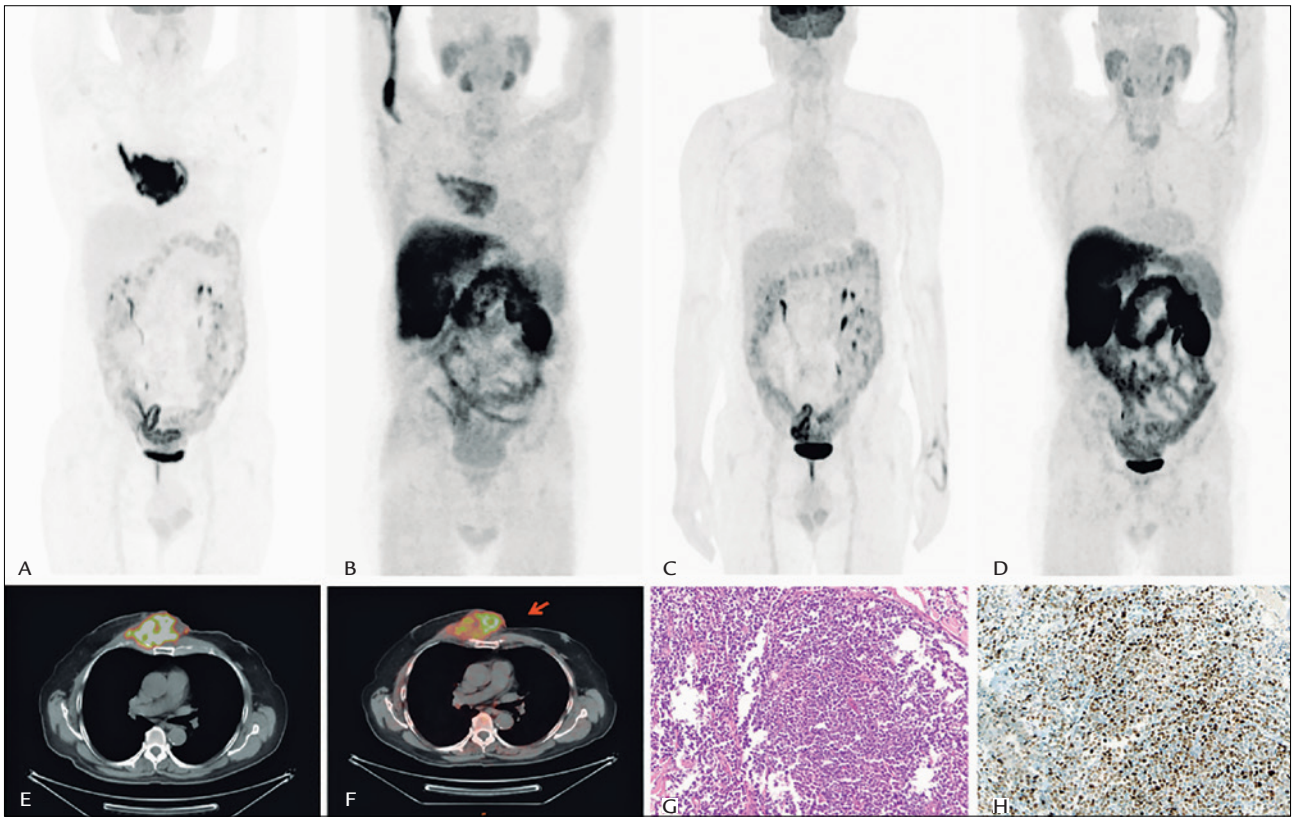

FIGURE 1 A 61-year-old man diagnosed with sternal cutaneous large B-cell non-Hodgkin lymphoma (NHL) (Panel G) underwent PET/CT scans pre- and post-therapy for tumor staging and assessment of response to treatment, respectively, using ^{18}F-FDG and ^{18}F-choline. The ^{18}F-FDG uptake (A and E) showed intense metabolic activity in areas of the tumor with high proliferative pattern (Panel H – strong and diffuse positivity for Ki-67). However, the uptake of ^{18}F-choline (B and F) was more intense in areas of lower glucose consumption of the tumor (arrow).

in the lesion site. Six weeks after chemoradiotherapy, post-treatment ^{18}F-FGD and ^{18}F-choline PET/CT scans were again performed and revealed no signs of radiotracer uptake, suggesting complete remission of the tumor (Figure 1C and D). Remission was confirmed in sixteen-month follow up with conventional imaging (CT).

^{18}F-FDG PET/CT imaging is a well-established imaging technique in clinical oncology.[7] One of the advantages of using this radiotracer is that it targets glucose phosphorylation, which is increased in malignant tumors due to overexpression of glucose membrane transporters and high glycolytic rate, creating positive tumor to background images.[8] Although it is considered as highly sensitive in tumor detection, current data indicate that the number of false positive images obtained from ^{18}F-FDG PET/CT is pointed as its most important limitation.[9]

As a non-specific radiotracer for malignant cells, the limitation of the test is based on the fact that ^{18}F-FDG uptake may be shown in areas with no carcinogenic behavior. Thereby, inflammation reaction (common in cancerous tissues), brown adipose tissue or even benign cell proliferation can create positive images, more likely to be misinterpreted as malignant cell proliferations.[9,10] A second significant limitation of this method relies on the fibrotic areas in post-therapy healing tissue to take up the radioactive glucose analogue even in cases of complete tumor remission, hence limiting treatment evaluation.[11]

As a complementary analysis, ^{18}F-choline PET/CT scans were obtained from a patient aiming to create more solid data regarding tumor profile, even though it is most commonly used in management of patients diagnosed with prostate cancer.[12] A previously published paper describes overexpression of choline kinase[12] in malignant cells, an enzyme responsible for phosphorylating choline into phosphatidylcholine, initiating the synthesis of cell membrane phospholipids.[12,13] Thereby, positive images are shown in response to increased cell proliferation in the lesion area, where more membrane formation is observed.[14,15]

Axial views obtained with ^{18}F-FDG and ^{18}F-choline PET/CT scanning show different radiotracer uptake in the same region of the tumor, possibly meaning diverse

cell differentiation. Areas of intense ^{18}F-FDG revealed low ^{18}F-choline uptake, and vice-versa, revealing a mismatch of metabolic and molecular biology of tumor behavior. On the other hand, both radiotracers were capable to evaluate response to chemoradiation therapy, confirmed with a long follow-up period.

^{18}F-FDG PET/CT scan is a valuable and established technique in NHL patient management. However, ^{18}F--choline can be a complimentary tool for tumor staging and assessment of therapeutic response in non-Hodgkin lymphoma. The images obtained in this study showing differential radiotracer uptake by cancer cells in distinct parts of the tumor depict differential metabolic behavior in the lesion. Non-^{18}F-FDG tracer can be used for targeted therapy and patient management.

Acknowledgments

The authors thank CNPq and FAPEMIG for financial support and Sofia Lage for text proofreading.

Conflict of interest

The authors declare no conflict of interest.

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Characteristics of training and motivation of physicians working in emergency medicine

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Summary

Introduction: Emergency medicine is an area in which correct decisions often need to be made fast, thus requiring a well-prepared medical team. There is little information regarding the profile of physicians working at emergency departments in Brazil.
Objective: To describe general characteristics of training and motivation of physicians working in the emergency departments of medium and large hospitals in Salvador, Brazil.
Method: A cross-sectional study with standardized interviews applied to physicians who work in emergency units in 25 medium and large hospitals in Salvador. At least 75% of the professionals at each hospital were interviewed. One hospital refused to participate in the study.
Results: A total of 659 physicians were interviewed, with a median age of 34 years (interquartile interval: 29-44 years), 329 (49.9%) were female and 96 (14.6%) were medical residents working at off hours. The percentage of physicians who had been trained with Basic Life Support, Advanced Cardiovascular Life Support and Advanced Trauma Life Support courses was 5.2, 18.4 and 11.0%, respectively, with a greater frequency of Advanced Cardiovascular Life Support training among younger individuals (23.6% versus 13.9%; p<0.001). Thirteen percent said they were completely satisfied with the activity, while 81.3% expressed a desire to stop working in emergency units in the next 15 years, mentioning stress levels as the main reason.
Conclusion: The physicians interviewed had taken few emergency immersion courses. A low motivational level was registered in physicians who work in the emergency departments of medium and large hospitals in Salvador.

Keywords: emergency medicine, physicians, motivation, clinical competence, advanced cardiovascular life support, Brazil.

Study conducted at Escola Bahiana de Medicina e Saúde Pública, Salvador, BA, Brazil

Article received: 6/30/2016
Accepted for publication: 7/9/2016

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http://dx.doi.org/10.1590/1806-9282.63.02.112

Introduction

Emergency medical care is designed to intercede in situations requiring quick or immediate decisions to reduce morbidity and mortality related to acute repercussions of systemic diseases or trauma.[1,2] For successful intervention in such situations, a qualified medical team, made up of professionals who are able to make quick decisions and apply effective trauma management and resuscitation techniques, is necessary.[3]

Care provided by the medical staff at the emergency department is a result of professional experience combined with a number of factors, namely proper training at an undergraduate or residency program, completion of immersion courses such as Basic and Advanced Cardiovascular Life Support (BLS and ACLS), motivation and perceived valorization.[4,5]

There is no information regarding these characteristics among emergency physicians in Brazil. This study

aimed to define the profile of doctors working in emergency departments of medium and large hospitals in a big Brazilian city in regard to professional training, motivation and expectations towards their work setting.

Method

Study design

This was a cross-sectional study in which the primary objective was to determine the professional profile of emergency physicians working in all medium and large hospitals in the city of Salvador, Bahia, Brazil. Data were obtained through interviews (questionnaire appliance), carried out between January and March 2012. The study was approved by the Institutional Ethics Committee of Santa Izabel Hospital.

Selection of participants

A questionnaire was applied to physicians working in the emergency services of all 25 medium and large size hospitals in the city of Salvador, the third largest city in Brazil.[6] Hospitals were classified according to the document "Concepts and Definitions in Health", according to which a medium sized hospital has 50 to 150 beds and a large one has 150 or more beds.[7] The number of hospital beds in every institution can be found at the National Database of Health Establishments.[8] All of the physicians gave consent to participate in this study. The Ethics Committee from each hospital was informed about this study. Only one institution refused to participate. Each institution provided a list with the names of all the physicians who were working in its emergency department and visits were made to each service to carry out interviews. The goal was to interview at least 3/4 of the emergency medical staff of each hospital, based on the time scales provided by the coordinators of the emergency services.

Data collection

The questionnaire applied was developed by the authors for the purpose of this research and included questions concerning medical education, graduate degrees and complementary courses, experience in emergency during and after the medical undergraduate program, dedication, and job satisfaction. The median of additional years doctors intended to work in emergency services was a quantitative variable. Interviews were carried out at the emergency department of each hospital by medical students previously trained to apply the questionnaire.

Primary data analysis

Data were expressed as means and standard deviations with Gaussian distribution, or median and interquartile interval when the distribution was not Gaussian. Frequencies were reported as absolute values and percentages. Chi-square test was used for categorical variables and the Mann-Whitney and Kruskal-Wallis for continuous variables. Data analysis was performed with SPSS software, version 13.0.

Results

A total of 659 physicians on duty at emergency departments of large and medium hospitals in Salvador were interviewed. This total corresponded to approximately 75% of the emergency physicians' estimate. We evaluated 24 hospitals, of which 11 (45.8%) were public, 6 (25%) were private, and 7 (29.2%) had both public and private care services (Table 1). Only one hospital refused to participate. All physicians evaluated gave consent to participation in the study.

The emergency physicians were usually young, presenting a median age of 34 years, ranging from 24 to 73 years old. There was no gender predominance. The median of working hours per week in emergency shifts was 24 hours (interquartile range [IQR]: 20-42 hours).

Most physicians (64.5%) considered they had good experience with emergency during the undergraduate program. Ninety-six physicians (14.6%) were medical residents working at residency off hours. About half declared to have specialist qualifications (Table 1).

The percentage of doctors who had already gone through BLS, ACLS and Advanced Trauma Life Support (ATLS) programs in the past two years was 5.2%; 18.4%; 11.0%, respectively (Table 2). ACLS training was the most frequent among the youngest physicians (p=0.001) (Table 3). When asked about what was the main reason for choosing the emergency department as their workplace, the majority chose "I like the activity" out of five alternatives (41.7%) (Table 4). Only 13.1% were fully satisfied with their job (Table 5) and 81.3% said they intended to stop working at the emergency department in the next 15 years, pointing out "excessive stress at work" as their main reason (Table 6). The median of additional years doctors intended to work in emergency services was 5 years (IQR: 2-8 years).

In relation to their self-evaluation as emergency physicians, 11.7% claimed they performed excellently. There was a higher frequency of physicians who considered their performance excellent in the group of doctors who had completed a residency program (14.2% against 1.6%, in the non-residency group; p<0.001). Among the physicians satisfied with their job, 20.9% rated their performance as excellent, against 14.4% in the group of physicians dissatisfied with their job (p<0.001).

TABLE 1 General characteristics of the emergency services and interviewed physicians.

Type of funding	Number of emergencies	Number of emergency physicians		
Public	11 (45.8%)	332 (50.4%)		
Private	6 (25%)	172 (26.1%)		
Public and private	7 (29.2%)	155 (23.5%)		
Total	24	659		
General data	**N / frequency**	**Minimum**	**Maximum**	**Mean (years)**
Age	656	24	73	37.7
Time after degree	656	0	42	12.4
Weekly hours in the ER	524	0	15	4.9
Specialist title: Yes	333 (50.7%)			
No	324 (49.3%)			
Master's degree	33 (5%)			
Doctoral degree	8 (1.2%)			
Sex: Male	330 (50.1%)			
Female	329 (49.9%)			
Completed Medical Residency Program				
Yes (one or more)	502 (76.2%)			
Internal Medicine	97			
Cardiology	32			
General Surgery	110			
Pediatric	95			
Gynecology–Obstetrics	82			
Other residency	235			
No residency	157 (23.8)			
Currently resident				
Yes	96 (14.6%)			
Internal Medicine	15			
Cardiology	6			
General Surgery	8			
Pediatric	10			
Gynecology–Obstetrics	8			
Other residency	49			
No resident	563 (85.4%)			

TABLE 2 Frequency of knowledge and major updating courses in emergency medicine taken in the last two years.

Course	n=659
ACLS	121 (18.4%)
ATLS	72 (11.0%)
BLS	34 (5.2%)

ACLS: Advanced Cardiovascular Life Support; ATLS: Advanced Trauma Life Support; BLS: Basic Life Support.

TABLE 3 Frequency of physicians certified in the major immersion courses in emergency medicine in the last two years by age.

Course	< 34 years (n=311)	≥ 34 years (n=345)	p-value
ACLS	73 (23.6%)	48 (13.9%)	0.001
ATLS	37 (12.0%)	35 (10.1%)	0.456
BLS	19 (6.1%)	15 (4.3%)	0.300

ACLS: Advanced Cardiovascular Life Support; ATLS: Advanced Trauma Life Support; BLS: Basic Life Support.

TABLE 4 Main reasons stated for working in emergency services.

Reason	n=659
I like the activity	270 (41.7%)
It is a job opportunity	145 (22.4%)
Good financial income	137 (21.2%)
I want to acquire experience	63 (9.7%)
Other reasons	32 (4.9%)
Did not respond	12 (1.8%)

TABLE 5 Degree of satisfaction of the physicians with their practice in emergency services.

Degree of satisfaction	n=659
Satisfied	86 (13.1%)
Neutral	473 (71.8%)
Dissatisfied	97 (14.7%)
Did not respond	3 (0.5%)

TABLE 6 Main reasons for not working in emergency in the next 15 years (more than one option could be chosen).

Reason	n=534
Stress	339 (63.7%)
Low remuneration	192 (36.2%)
Inappropriate working conditions	283 (53.4%)

When analyzing the main reason for working in the emergency department, the frequency of the answers "It's a job opportunity" and "Good financial income" was higher in the group of emergency physicians who were dissatisfied with their job (30.4% and 35.1%, respectively). On the other hand, doctors in the "satisfied" group chose "I like the emergency practice" more often (52.4%). The differences were all statistically significant ($p<0.001$).

Among the physicians who were satisfied, 64.0% intended to stop working in emergency medicine in the next 15 years, compared to 93.8% of the dissatisfied doctors and 81.8% of those who were neutral and declared themselves neither satisfied nor dissatisfied ($p<0.001$). Regarding the reasons for stopping working in emergency medicine in the next 15 years, 41.8% of the satisfied, 64.2% neutral and 75.8% dissatisfied physicians reported "stress at work" ($p<0.001$) (Table 7).

Comparing to private and mixed services, the physicians of exclusively public emergency rooms were older, more frequently specialists, less satisfied and less trained in ACLS (Table 8).

Discussion

The aim of this study was to evaluate the general and professional profile of physicians working in emergency departments in the third largest city in Brazil. There was a predominance of young doctors working in the emergency department, similar to what Rosenbach et al.[9] had described. In their report, emergency physicians were younger and less qualified than physicians from other specialties.

Although male doctors are still predominant in Brazil,[10] we found a balanced gender distribution. This may reflect a transition of gender predominance that is seen in Brazil among the youngest physicians.[10]

Half of the physicians had finished their residency program and almost 15% of the professionals were still undergoing their residency. Furthermore, the low frequency of involvement in immersion courses is surprisingly remarkable, as the majority of the most traditional courses focus on various aspects of the emergency practice. Analyzing the results by age, we observed that younger physicians engaged in ACLS courses more often than older ones, which might reflect greater motivation for continuing learning and training among those who are initiating their professional career.

A cross-sectional study conducted between 2003 and 2004 in Salvador, Brazil, evaluated knowledge about cardiopulmonary resuscitation among emergency physicians in public and private hospitals. This report showed that 70.5 and 66.9% of the doctors had never taken ACLS or ATLS courses, respectively.[11]

In our study, 64% of the emergency physicians reported they had good experience in emergencies during their undergraduate program. Another study, conducted with final year medical students from the Faculty of Medical Sciences of the Rio de Janeiro State University showed that 93.7% of the students had done at least one extracurricular training course during their degree. Emergency medicine and intensive care were chosen more frequently than other areas, because students felt they needed more training in these settings despite their regular undergraduate training.[12] In Israel, 65% of the students from the Hebrew University – Hadassah Faculty of Medicine considered "diagnosis and management of medical emergencies" as one of the most deficient points of their medical training.[13] In Iran, on the other hand, a study involving a questionnaire answered by students from Tehran University of Medical Sciences – School of Medicine showed that 69.4% of the students classified their training in medical emergency as "good" or "very good."[14]

In Brazil, in contrast with different countries around the world, there are few residency training programs in

TABLE 7 Differences regarding the reasons for not working in emergency by degree of satisfaction with the job (more than one answer could be chosen).

	Fully satisfied	Partially satisfied	Dissatisfied	p-value
Stress	23 (41.8%)	247 (64.2%)	69 (75.8%)	<0.001
Low remuneration	5 (9.1%)	128 (33.4%)	58 (63.7%)	<0.001
Inappropriate working conditions	7 (12.7%)	201 (52.5%)	74 (81.3%)	<0.001

TABLE 8 Comparisons related to the type of emergency service.

	Public	Private or public and private	p-value
Age			0.004
< 34 years	138 (41.8%)	173 (53.1%)	
≥ 34 years	192 (58.2%)	153 (46.9%)	
Mean formed time (years)	15	10	<0.001
Specialist title frequency	191 (57.9%)	142 (43.4%)	<0.001
Degree of satisfaction			0.001
Satisfied	36 (10.9%)	50 (15.3%)	
Neutral	229 (69.4%)	244 (74.8%)	
Dissatisfied	65 (19.7%)	32 (9.8%)	
Knows ACLS	248 (75.2%)	303 (92.7%)	<0.001

ACLS: Advanced Cardiovascular Life Support.

Emergency Medicine. Currently in Brazil there are only 27 residency programs in Emergency Medicine, distributed in nine states. This number falls far short compared, for example, with specialties such as Internal Medicine or General Surgery, with 287 and 356 programs in all Brazilian states, respectively.[15] In the United States, there are approximately 5,000 emergency departments with 25,000 physicians practicing in these centers. Currently more than 2,700 physicians are being trained in the 132 Emergency Medicine Residency Training Programs approved by the US Accreditation Council for Graduate Medical Education (ACGME). Each year these programs award degrees to approximately 800 physicians that are eligible to become certified as Emergency Medicine specialists. Emergency Medicine has been and continues to be one of the most competitive specialties for medical student applicants.[16]

Despite stating that the main reason for working in the emergency room was that they liked the activity, most physicians expressed their intention to stop working in this field in the next 15 years. This fact highlights the importance to study the profile of the professionals who are working in emergency services. Few professionals seemed to have plans to pursue a career in emergency medicine, even among those fully satisfied with this practice, and only some of them reported to be satisfied with their job. Curiously, the main reasons for working in the emergency department ("good financial income" and "job opportunity") were significantly associated to dissatisfaction with the job as an emergency physician.

In a study with physicians from the American Board of Emergency Medicine (ABEM) about job satisfaction in emergency practice, 23.1% of the physicians reported an intention to stop working in emergency medicine in five years time.[17] Many factors may influence the ceasing of medical emergency practice, such as the great number of patients seen per hour, the stress involved and burnout syndrome.[18,19] A Canadian cross-sectional study showed that emergency physicians are emotionally exhausted, with high levels of depersonalization and relatively low levels of personal accomplishment.[20]

A lower level of satisfaction was associated with practice in public emergency services. A study in Turkey that assessed burnout in public versus private emergency rooms also found that in the public sector, work locations, false accusations, occupational injuries and diseases, work-related permanent disabilities, and organizational support have significantly influence in self-reported perceptions of well-being ($p<0.05$).[21]

There were some limitations in our study. The questionnaire was elaborated and applied by the authors of the article. However, no validated instrument about this theme was found in the medical literature to be used for the purposes of the present study. It was not possible to interview the entire population of physicians working in emergencies from Salvador. This is probably because the

population of doctors working in emergency services changes constantly, and has a fast turnover, associated to the fact that most physicians work regularly in areas other than emergency with occasional shifts in the emergency department, without an actual employment bond in the area. Even though we used a convenience sampling, we believe that our findings likely reflect the characteristics of our target-population, since more than 75% of the emergency physicians of all large and medium size hospitals were interviewed. It was not possible to compare a group that received specific training in emergency medicine with another that did not, because there were no specific training programs in the country. Some factors such as safety at work and provision of materials for an adequate job could have been included in the questionnaire in order to assess their influence on satisfaction of emergency physicians, for example. Additionally, the alternatives in some multiple choice questions might not reflect the exact thoughts of each individual.

Conclusion

We found that the emergency physicians of large and medium-sized hospitals in the city of Salvador are relatively young, without gender predominance. They have little training in emergency immersion courses, and need to be more motivated to practice their profession fully in emergency departments. This information could be extrapolated for other countries that have emergency services working under similar conditions of funds and labor, such as other developing countries or those located in Latin America.

Conflict of interest

The authors declare no conflict of interest.

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A prospective randomized trial comparing patent blue and methylene blue for the detection of the sentinel lymph node in breast cancer patients

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Summary

Introduction: Methylene blue is more widely available and less expensive than patent blue, with an apparently lower risk of anaphylaxis.
Objective: The two dyes were compared regarding detection of the sentinel lymph node (SLN).
Method: A prospective, randomized trial involved 142 patients with invasive breast carcinoma. Sixty-nine (49.3%) assigned to patent blue (group A) and 71 (50.70%) to methylene blue (group B). Thirty-five patients (25.0%) were clinical stage III or IV; 55 (38.7%) had axillary lymph nodes affected; and 69 (49.3%) underwent neoadjuvant chemotherapy. Two patients were excluded because the dye type was not recorded.
Results: Patients and tumor characteristics were similar in both groups. SLNs were identified in 47 women (68.1%) in group A and 43 (60.6%) in group B (p=0.35). SLNs were affected in 22 cases (51.2%) in group A and 21 (48.8%) in group B (p=0.62). The SLN was the only node affected in 12 cases (54.5%) in group A and six (33.3%) in group B (p=0.18). The time and degree of difficulty involved in identifying the SLN were similar in both groups. There were no complications or allergies.
Conclusion: Methylene blue performed as well as patent blue in identifying the SLN in breast cancer patients.

Keywords: breast cancer, sentinel lymph node, patent blue, methylene blue, randomized controlled trial.

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Article received: 5/16/2016
Accepted for publication: 5/31/2016

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Funding: Partially funded by the Goiás State Foundation for the Support of Research (FAPEG), grant 01/2007

http://dx.doi.org/10.1590/1806-9282.63.02.118

Introduction

Prior to the first studies on sentinel lymph nodes, axillary lymph node dissection was considered the standard treatment for patients with early stage breast cancer.[1,2] Indeed, the extent of axillary involvement is one of the most important independent prognostic factors for tumor recurrence and patient survival.[2,3] Nevertheless, dissection of the entire axillary lymph node chain results in greater morbidity, and a considerable percentage of the patients previously submitted to this procedure were later found to have no axillary metastasis, with the intervention therefore having been unnecessary.[1]

The introduction of the sentinel lymph node biopsy was one of the greatest advances in the surgical treatment of breast cancer and has proved an excellent predictor of axillary involvement in initial tumors.[4,5] Since sentinel lymph node biopsy involves fewer risks and less morbidity, it has gained followers worldwide and is currently the standard treatment for axillary management in breast cancer patients.[6] This method enables axillary involvement to be diagnosed with

good sensitivity. Therefore, complete dissection of the axillary lymph nodes became restricted to those cases in which metastases are detected in the sentinel lymph node.

When only micro-metastases are found in the sentinel lymph node, complete dissection is considered unnecessary.[7] In the case of conservative surgery with conventional radiotherapy, standard axillary dissection can be avoided when only one or two lymph nodes are affected.[8] In the case of mastectomy or when up to three lymph nodes are affected, axillary radiotherapy can be performed, with lower morbidity rates compared to axillary dissection.[9,10]

Some investigators have preferred the use of nuclear medicine techniques to identify the sentinel lymph node due to the greater simplicity of those techniques compared to the use of dyes.[5,11] With dyes, the surgeon may require slightly more training and the learning curve may be steeper.[12] After the initial training period, however, the sentinel lymph node identification rate with dyes tends to be similar to that obtained with nuclear medicine techniques, reaching 98% in some more recent reports.[13] Furthermore, once the sentinel lymph node is identified, accuracy is the same irrespective of the method used and the lymph node detection rate.

The principal problems involved in radioactive tracer techniques are their technological complexity and high costs.[5] In this respect, the use of dyes is still the most economically viable alternative, principally in public healthcare services with few resources, a common scenario in developing countries. The cost of the same procedure may be much lower with the use of dyes compared to the use of radioactive tracers.

Different vital dyes have been used to identify the sentinel lymph node: patent blue, isosulfan blue and, less commonly, methylene blue.[11,13] Although methylene blue is more readily available and considerably less expensive than the others, some authors claim that it diffuses more rapidly in peripheral tissues, staining a larger portion of the breast with the blue dye and, to a certain extent, hampering the procedure.[14-16] Other authors have reported similar accuracy and sentinel lymph node detection rates with methylene blue and with patent blue.[13,17] There appears to be a lower risk of anaphylaxis with methylene blue compared to the other dyes.[18] Therefore, the objective of the present study was to compare the detection rate and accuracy of two different dyes, methylene blue and patent blue, for the identification of the sentinel lymph node in patients with breast cancer.

Method

In this prospective randomized study, 142 patients with a diagnosis of invasive breast carcinoma were included. The patients were receiving care within the Breast Program at the University of Goiás Teaching Hospital. The institutional review board approved the study. All the patients signed an informed consent form.

The participants were scheduled to undergo sentinel lymph node biopsy or complete axillary lymph node dissection. The women were enrolled irrespective of their clinical staging, comorbidities or previous treatment (previous surgeries, chemotherapy or radiotherapy). A total of 69 patients (49.3%) received a 2-mL injection of patent blue (Group A) and 71 (50.7%) a 2-mL injection of 1% methylene blue (Group B). Two patients were excluded from the analysis because the type of dye used had not been recorded appropriately.

For the calculation of sample size, the factors taken into consideration were the possibility of a sentinel lymph node detection rate of 95% with patent blue[11,19] and a hypothetical difference of 20% less in the detection rate with the use of methylene blue. In fact, the few published studies available comparing the two methods failed to show any difference between them.[15,20] Considering a confidence level of 5% and a power of the test of 80%, a total of 116 patients would be required, divided into the two groups. To compensate for any possible losses, 142 patients were enrolled to the study.

The patients admitted to the study underwent various different steps that consisted of a detailed bilateral breast examination, laboratory tests including full blood count, alkaline phosphatase, aspartate aminotransferase (AST) and alanine transaminase (ALT), chest X-ray (posterior-anterior and lateral), ultrasonography of the upper abdomen, bone scintigraphy, bilateral mammography and the preoperative examinations appropriate for each individual case.

Mastectomy or quadrantectomy was performed in accordance at the discretion of the attending physician, together with sentinel lymph node biopsy. Level I, II and sometimes level III axillary lymphadenectomy was performed when axillary involvement was found immediately prior to surgery, either clinically, by palpation or from imaging tests. The patients were previously randomized using a computer-generated randomization system to the use of patent blue or methylene blue dye. According to the randomization group, 2 mL of 2.5% sterile patent blue dye or 2 mL of sterile methylene blue dye were injected into the peritumoral or periareolar region. Next, the site was massaged for 5 minutes. Sentinel lymph nodes were defined as all the lymph nodes stained blue or when the afferent lymphatic vessels were blue (Figure 1). Clinically suspect lymph nodes were not considered sentinel lymph nodes.

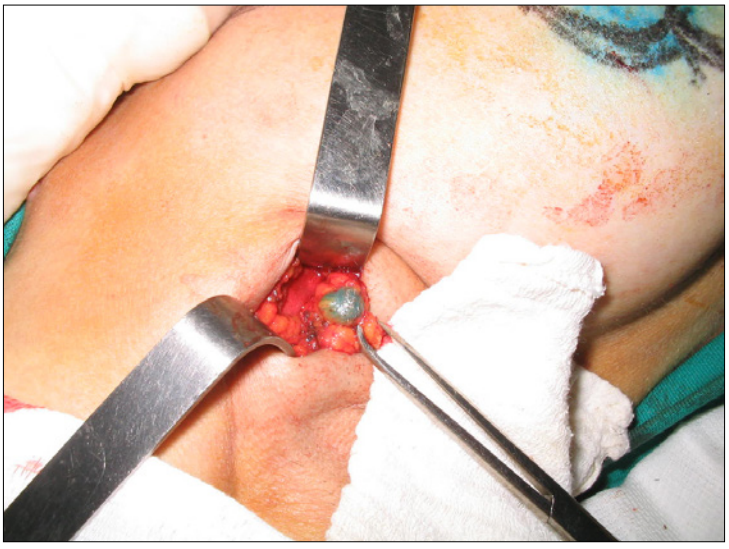

FIGURE 1 Sentinel node in the armpit identified by means of the color, after the periareolar injection of the blue dye.

The surgical specimen was sent to pathology and histological confirmation was reached by frozen section biopsy. The sentinel lymph nodes were submitted to histological evaluation separate from the rest of the lesion and from the other lymph nodes.

The data were collected on a form specifically designed for this study and entered into a database. SPSS statistical software package, version 15.0, was used for the statistical analysis. Chi-square test was used to compare accuracy between the two groups using the formulae for the comparison of two independent samples described by Galen & Gambino.[21] P-values < 0.05 were considered statistically significant.

Results

There were no statistically significant differences between the two groups with respect to the characteristics of the patients or their tumors (Table 1). The type of treatment given and the immediate results were similar in both groups (Table 2). The time required to identify the sentinel lymph node and the degree of difficulty encountered were similar in the two groups. The sentinel lymph node identification rate was similar in both groups, as were the other parameters compared (Table 3).

Discussion

The use of radioactive isotope techniques to identify the sentinel lymph node in patients with breast cancer has been limited, mainly because a considerable proportion of healthcare services cannot afford their costs. Dyes are widely used with acceptable results, both in association with a radioactive isotope or on their own.[22] However, the sentinel lymph node identification rate with dyes is largely dependent on the experience of the medical team. Giuliano et al. reported an increase in the sentinel lymph node identification rate with patent blue dye from 66 to 94% as their experience increased.[11,19]

Although patent blue and isosulfan blue are the dyes most commonly used in the sentinel lymph node technique, some groups have reported success with the use of methylene blue.[17,18] The difference in cost between the dyes is considerable and methylene blue is much more readily available in different hospitals. The cost of methylene blue may represent as little as 3% of the cost of patent blue or isosulfan blue.[23] For healthcare institutions in developing countries, particularly those that depend on public funding, this cost reduction may make the use of the sentinel node technique more easily available.

The risk of allergic reactions ranges from 1 to 2% with patent blue and isosulfan blue, with these reactions being severe in some cases.[17,18] Up to the present moment, there have been no reports of allergies with methylene blue. As the popularity of the sentinel lymph node technique increases, preference for the use of methylene blue rather than the other dyes may avoid many undesirable and potentially fatal allergic reactions.[13] Furthermore, compared to the other dyes, methylene blue interferes less with oximetry.[24] Nevertheless, a case has been reported in the literature of a pulmonary edema possibly related to the use of methylene blue for the detection of the sentinel lymph node.[25] Another advantage of methylene blue is the possibility of being able to use it during pregnancy, which is not the case with the other dyes.[26]

TABLE 1 Characteristics of the patients and tumors.

	Patent blue	Methylene blue	p-value
Age (years)[a]	51.00 (+11.48)	52.82 (13.44)	0.39
Size of the tumor (mm)[b]	35.00 (25.00–50.00)	33.00 (25.00–50.00)	0.78
Clinical staging[c]			0.34
I	8 (11.6%)	11 (15.5%)	
IIa	28 (40.6%)	30 (42.3%)	
IIb	14 (20.3%)	14 (19.7%)	
III	17 (24.6%)	15 (21.1%)	
IV	2 (2.9%)	1 (1.4%)	
Clinical involvement of axillae	32 (46.4%)	22 (31.0%)	0.06
Histological grade[c]			0.90
I	13 (23.6%)	16 (28.6%)	
II	32 (58.2%)	28 (50.0%)	
III	10 (18.2%)	11 (19.6%)	
Skin color[d]			0.73
White	27 (39.1%)	32 (45.7%)	
Black	7 (10.1%)	6 (8.6%)	
Brown	35 (50.7%)	32 (45.7%)	
Histological type[d]			0.56
Invasive ductal carcinoma	61 (88.4%)	60 (84.5%)	
Invasive lobular carcinoma	3 (4.3%)	3 (4.2%)	
Others	5 (7.3%)	8 (11.3%)	
Multicentric tumor[d]	13 (18.8%)	15 (21.4%)	0.70

[a]Mean (± standard deviation), Student's t-test.
[b]Median (interquartile range), Mann-Whitney U test.
[c]n (%), Mann-Whitney U test.
[d]n (%), Chi-square test, with or without Yates' correction, or Fisher's exact test.

TABLE 2 Characteristics of the techniques and of the treatment.

	Patent blue	Methylene blue	p-value
Lymph node dissection[a]	15.2 (±6.8)	15.9 (±8.5)	0.67
Lymph nodes affected[b]	1.0 (0.0-2.5)	0.0 (0.0-5.0)	0.93
Previous open biopsy[c]	15 (21.7%)	21 (29.6%)	0.29
Neoadjuvant chemotherapy[c]	39 (56.5%)	47 (66.2%)	0.24
Conservative surgery[c]	37 (53.6%)	35 (49.3%)	0.87

[a]Mean (± standard deviation), Student's t-test.
[b]Median (interquartile range), Mann-Whitney U test.
[c]n (%); Chi-square test.

TABLE 3 Results of sentinel lymph node investigation.

	Patent blue	Methylene blue	p-value
Detection of sentinel lymph node	47 (68.1%)	43 (60.6%)	0.35[a]
Involvement of sentinel lymph node	22 (51.2%)	21 (48.8%)	0.62
Sentinel lymph node was the only node affected	12 (54.5%)	6 (33.3%)	0.19[a]
Number of sentinel lymph nodes	1.0 (0.0-2.0)	1.0 (0.0-2.0)	0.43[b]
Time until detection of sentinel lymph node (minutes)	14.0 (4.0-45.0)	11.0 (1.0-31.3)	0.34[b]

[a]n (%); Chi-square test for trend (Mann-Whitney U test).
[b]Median (interquartile range); Mann-Whitney U test.

There have been reports of a few cases of skin necrosis and fat necrosis following injection of different dyes; however, no complication of this type was found in the present study.[27,28]

In our study, the sentinel lymph node detection rate was 68.1% in the patent blue group and 60.6% in the methylene blue group, with no statistically significant difference between the two groups. This detection rate may appear low, but this can be explained by the large number of locally advanced tumors, of cases in which axillary involvement was present and of cases in which neoadjuvant chemotherapy had been performed. Furthermore, physicians undergoing training are given the opportunity to start learning a new specialty in this university teaching hospital. Studies with patent blue alone have shown a detection rate of 60 to 75% at the beginning of the learning curve.[19,29]

For various reasons, we decided that all patients with invasive carcinomas should be included in the study, even cases lacking conventional indication for sentinel lymph node. This would allow a greater number of cases to be included, providing the team with a better training opportunity and conferring greater statistical power to the analysis. Since the patients were allocated randomly into the groups, there does not appear to be any type of selection bias in comparing the efficacy of each method. Once the sentinel lymph node was identified, the accuracy of the different dyes appears to be similar to rates published in the literature.[5,30] The degree of technical difficulty appears to be the same in the two groups.

Since the physical examination and imaging tests performed may raise false suspicions, a biopsy of the sentinel lymph node was performed rather than percutaneous lymph node biopsy, and the suspect lymph node was removed separately if not simultaneously, followed by intra-operative evaluation by cytology or histology. Due to the strict study methodology, a lymph node that was considered suspect from a clinical point of view but that had not been stained was not considered a sentinel lymph node.

Use of sentinel lymph node biopsy in clinical practice, despite still involving considerable morbidity, provides better results for the patients than complete axillary dissection.[31] In certain cases, such as those with larger tumors, following neoadjuvant chemotherapy, or when there is prior axillary involvement, identification of the sentinel lymph node is more difficult and there is a greater risk of false-negative findings. The American College of Surgeons Oncology Group (ACOSOG)'s Z1071 trial and the Sentinel Neoadjuvant (SENTINA) study both reported errors in identifying the sentinel lymph node that exceeded the limit considered acceptable following chemotherapy.[32,33] It is not yet known whether slightly more false-negative findings could translate into unfavorable oncological results such as a greater incidence of recurrence of the disease and higher mortality. In those studies, the best and most acceptable results were obtained when two types of markers were used, a dye in association with radioactive technetium, and when more than two lymph nodes were removed.

Despite the financial limitations imposed by the healthcare system and by the teaching hospital, we believe that this study represents an important contribution towards being able to offer an alternative to the use of nuclear medicine even when circumstances are unfavorable such as in this study population.

Conclusion

Methylene blue can be used as a substitute for patent blue in sentinel lymph node biopsies, with no increase in the complication rate or in the degree of technical difficulty, and with the added advantage of lower cost.

Resumo

Estudo randomizado prospectivo comparando o azul patente ao azul de metileno para a detecção do linfonodo sentinela em pacientes com câncer de mama

Introdução: O azul de metileno é mais facilmente encontrado para comercialização e a um preço menor que o azul patente. Parece ainda haver menor risco de anafilaxia.
Objetivo: Comparar a taxa de detecção do linfonodo sentinela com o azul patente e com o azul de metileno.
Método: Foram incluídas, de forma randomizada e prospectiva, 142 pacientes com diagnóstico de carcinoma mamário invasor, que consentiram em participar livremente do estudo. Foram injetados 2 mL de azul patente (grupo A) em 69 (49,3%) mulheres e de azul de metileno (grupo B) em 71 (50,70%), em localização periareolar ou peritumoral, seguido de 5 minutos de massagem. Trinta e cinco (25,0%) apresentavam estadiamento clínico 3 ou 4, e 55 (38,7%) apresentavam a axila clinicamente comprometida. Sessenta e nove (49,3%) fizeram quimioterapia neoadjuvante. Duas pacientes não tinham anotação do corante utilizado e foram excluídas.
Resultados: Os dois grupos apresentaram características das pacientes e dos tumores semelhantes. Foram detectados linfonodos sentinela em 47 (68,1%) mulheres no grupo A e em 43 (60,6%) no grupo B (p=0,35). Havia linfonodos sentinela comprometidos em 22 (51,2%) casos

no grupo A e em 21 (48,8%) casos no grupo B (p=0,62). O linfonodo sentinela foi o único gânglio comprometido em 12 (54,5%) casos no grupo A e em seis (33,3%) casos no grupo B (p=0,18). O tempo e o grau de dificuldade para identificação do linfonodo sentinela foram semelhantes nos dois grupos. Não houve relato de complicações ou de alergia em nenhum dos grupos.

Conclusão: A utilização do azul de metileno para a identificação do linfonodo sentinela em pacientes com câncer de mama apresenta resultados semelhantes aos do azul patente.

Palavras-chave: câncer de mama, linfonodo sentinela, azul patente, azul de metileno, ensaio clínico randomizado.

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Overweight and obesity in preschoolers: Prevalence and relation to food consumption

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Summary

Objective: To determine overweight and obesity prevalence in preschool children from public education, and to determine their relation to food consumption.
Method: Cross-sectional study with children aged between 2 and 5 years, of both sexes, enrolled at municipal day care centers. Socioeconomic, demographic and anthropometric data were collected, in order to calculate the body mass index (BMI) for age. Data on food consumption were assessed using a Food Frequency Questionnaire. χ^2 test, Kruskal-Wallis test, Student's t-test and Pearson's correlation were used at a significance level of 5%.
Results: Of 548 children, 52% were male, with mean age of 4.2 years old. Most families had incomes between 1 and 2 minimum wages (59.7%), in addition to 10 years (mothers) of education. Anthropometric parameters did not differ significantly between sexes. According to the BMI-for-age, it was found that most of children were well-nourished (85.2%), 8.2% had the risk of becoming overweight, and 4.2% were overweight. The most consumed foods were: rice (100%), beans (99.4%), bread (98.5%), fruit (98.5%), red meat (97.1%), butter and margarine (95.4%), biscuits, cakes and sweet pies (94.1%), dairy products (94.1%), chocolate milk (91.7%), and soft drinks (90.2%). Consumed foods that were strongly correlated ($r > 0.7$) to the risk of/excess weight were, as follows: bread; biscuits, cakes, sweet pies; dairy products; chocolate milk; sausages.
Conclusion: There was low prevalence of overweight and absence of obesity among the population assessed. The risk of overweight was greater among girls. Data from the study showed deviations in food consumption.

Keywords: overweight, obesity, preschooler, food consumption.

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Article received: 5/31/2016
Accepted for publication: 6/20/2016

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http://dx.doi.org/10.1590/1806-9282.63.02.124

Introduction

The change in the nutritional profile of Brazilian children due to the nutritional transition has revealed the increasing prevalence of overweight and obesity among children, in parallel to the reduction of nutritional deficits, and this situation has become alarming due to rising indicators.[1]

Estimates by the World Health Organization (WHO) suggest that excess weight affects around 5 million children aged under 5 years worldwide.[2] The Family Budget Survey (POF, in the Portuguese acronym) 2008-2009 showed an important increase in the number of obese children in Brazil, with a prevalence of 32.8% overweight and 16.2% obesity among children up to 5 years of age.[3]

The growing trend of overfeeding, overweight and obesity means the prevention of these outcomes is of the utmost priority in order to prevent complications in adult life, considering the multifactorial nature of this condition.[4,5]

The first years of life are sensitive to nutritional changes and metabolic disorders, and it is a critical period for establishing habits and behaviors that will influence both growth and child development.[6,7]

Therefore, assessment of the composition of the diet of preschoolers has become an extremely relevant point,

considering the impact that consumption can have on the overall health of this age group, preventing imbalances both due to deficiency diseases and dietary excess.[8]

Faced with the increasing prevalence of overweight and obesity, as well as changes in food consumption among preschool children, it is necessary to obtain representative data for this population, which is exposed to this risk the most often, in order to enable further follow-up, monitoring and intervention measures, whenever these become necessary. Therefore, this study aimed to verify the prevalence of overweight and obesity in preschoolers at public education institutions in Teresina, capital of the Brazilian state of Piauí, and its relationship to food consumption.

Method

This is a cross-sectional and analytical study, in which we assessed preschoolers of both sexes aged 2 to 5 years and enrolled in the Municipal Public Education Network of Teresina, in the Brazilian state of Piauí, from the four census regions of the city. The day care centers were defined by means of drawing lots, as well as the sample population.

For calculation of the sample size (n), a 10% prevalence of overweight and obesity was adopted, as observed in a study with Brazilian children of low socioeconomic status,[9] obtaining n = 548 children, considering a margin of error (e) of 5% and a confidence level of 95%.

To begin the collection of data, the approval of the Ethics Committee of the Federal University of Piauí (Report No. 94772/12), the Department of Education and the parents/guardians of the students was obtained by signing the informed consent form, in addition to obtaining the Consent of Participation of Person as a Subject.

The inclusion criterion was the absence of physical or motor restrictions that could impair the collection of anthropometric data.

We collected and recorded data relating to the general conditions and health of the child, such as age, sex, birth weight, current height and weight, time attending day care, the occurrence of diseases, control of parasitosis and the use of vitamins and medicines, socioeconomic data (level of education and parental occupation, household income, number of family members, number of rooms in the house, basic sanitation and type of housing) as well as an anthropometric assessment with measurement of body weight and stature. The anthropometric measurements were determined using standard techniques.[10,11] To measure the weight, a pre-calibrated Wiso W910 ultra slim digital scale with capacity for 150 kg and graduation in units of 100 g was used, and a WCS® stadiometer, with measurements in triplicate.

The weight and height data were used to calculate the body mass index (BMI), which was then adjusted for age in the Z-score using the WHO's growth curves.[2] The WHO criteria were used to classify the nutritional status,[12] as well as that adopted by the Brazilian Ministry of Health,[13] which was BMI for age, using the Anthropometric Program ANTHRO.[14]

Food consumption of the preschoolers was verified using a previously validated qualitative Food Frequency Questionnaire (FFQ) completed by the child's guardian or parent.[15] Foods were categorized with the following consumption frequencies: consumes versus does not consume. The "consumes" category included consumption when this occurred daily, weekly or fortnightly. The "does not" category included monthly consumption or just non-consumption. The questionnaire included foods consumed regionally and the usual food for this population.

For the statistical analysis a database was created using Statistical Package for the Social Sciences, software version 17.0.[16] The results were presented using simple and cross-frequency tables, with absolute values, percentage, means and standard deviations, when necessary. In order to verify the association, Chi-square (χ^2) test was applied on the nominal variables and the Kruskal-Wallis test was used to test differences in proportions. Student's t-test was applied to the numerical variables (age, weight, height). Pearson correlation was used in the Food Frequency in relation to nutritional state. Significance of 5% was adopted for all tests ($p<0.05$).[17]

Results

The sample was composed of 548 preschoolers aged between 2 and 5 years originating from day care centers located in the city's four zones. Fifty-two percent of the participants were male and 48% female, without a significant difference in the χ^2 test, as per Table 1.

It can also be noted in Table 1 that the average age was 4 years and 2 months, and there was no significant difference ($p=0.874$) between age and sex. The birth weight was around 3.169 kg in the group of children studied and the average time enrolled at the school was 1 year and 6 months.

With respect to the current weight, boys presented a mean of 17.1 kg, and the girls a mean of 16.7 kg. The mean current height among the boys was 105 cm and 104 cm among the girls. No significant difference was observed in the height and weight in relation to sex ($p>0.05$).

The average was five people per family. Regarding the mother and father's level of education, the average time of education of the mother was around 10 years, while the fathers were formally educated for 9 years on average. The stratum between 9 and 12 years of study presented the highest frequency in both variables related to level of education.

As for family income, most families (59.7%) were in the stratum between 1 and 2 minimum wages, with only 10.2% families above this level, meaning that this population was characterized as being low income, a profile which is to be expected of preschoolers originating from public institutions.

Table 2 shows the results in absolute figures and percentage referring to the housing conditions of the children participating in the study. The data showed an average of six rooms per residence which, when making a ratio of rooms to people, shows an approximate average of one room per person.

With respect to the type of housing, masonry/brick houses were predominant (95.8%), and only 4.2% of houses were made of rammed earth. In relation to power supply, 100% of dwellings had electricity. The water supply from the public network was 97.8% indoors and only 2.2% outside of the home. The result also showed that 100% of homes were served by official garbage collection. It was noted with respect to sanitation that 88.7% of households were connected to public wastewater network and only 11.3% were served by a septic tank. In relation to the access roads, 53.5% of the families lived in regions with streets that were not paved.

Food consumption of the preschoolers is shown in Table 3 as percentages based on the responses given by the parents/guardians with regards to the foods consumed the most by the children.

We noted that the daily consumption of fruits and vegetables, chicken, eggs, beef liver, giblets and seafood was infrequent, which characterizes the presence of a monotonous diet, deficient in essential nutrients such as proteins of high biological value, vitamins, minerals, and unsaturated fatty acids essential for this growth phase.

We did not note the consumption of fast food in the group, probably because of the less favorable economic stratum and/or the age group in which they were found, which reduces access to this type of food.

Table 4 shows the results of the correlation between food consumption and nutritional status. It was observed that foods with a moderate positive correlation with risk of/excess weight were: soft drinks, butter and margarine, fried foods, coffee, pasta and artificial juices. Meanwhile, milk and dairy products, cookies, cakes, pies, sausages, breads and chocolate milk showed a strong correlation with risk and excess weight.

Discussion

In relation to birth weight, a similar study found a frequency of overweight that was 64% higher in children who had a birth weight ≥ 3.9 kg.[18] This suggests that the mean (3.169 kg) obtained in this study may have been a protective factor for the overweight condition.

A study with 1,544 children of public day care centers revealed a mean of 4.6 persons per household,[19] a value close to that observed in our study. However, it indicated six years of maternal schooling and five years for the father, lower values than those found in our research.

The years of education of the mother were closer to the level verified in research assessing 132 children at day care centers in Rio de Janeiro, in the Brazilian state of Rio de Janeiro, which obtained a mean of 8.1 (± 3.05) years of schooling.[20] However, this was well above that reported in other studies assessing the prevalence and determinants of overweight among children and adolescents in the Brazilian state of Pernambuco, where the mean was four years of study for mothers.[21]

In our case, the time of formal education can be considered as relatively positive, given that we studied a low-income group, which creates the expectation of lower educational levels. There are discrepancies in the literature with regard to maternal education and the occurrence of overweight and obesity among children, given that such schooling increases the chances of parents entering the labor market, leading to increased family income. On the other hand, it is believed that a higher level of education can contribute by creating greater concern with the health of the child, including the encouragement of physical activity and consumption of healthier food, in addition to facilitating access to better quality foods.[22]

It is possible to note a positive association between the mother's level of education being greater than or equal to seven years of study and the occurrence of overweight among preschoolers.[18] A study of 1,187 schoolchildren in Divinópolis, state of Minas Gerais, Brazil, showed that the children of mothers with more than eight years of study had a 1.62 (1.19-2.19) times greater chance of being overweight than those whose mothers had eight years of study or less.[23]

Several studies have shown that in economically less developed regions/states the proportion of obesity rises with increasing income,[21,24,25] which may justify the absence of obesity among the target population researched. Corroborating these results, a study with children in this age

TABLE 1 Characterization of the preschool children studied.

Variables	N	%	Mean (SD)	Statistics
Sex				
Male	285	52.0		NS*
Female	263	48.0		
Age (years)				
Male	285	52.0	4.2 (1.2)	NS†
Female	263	48.0	4.1 (1.1)	
Birth weight (g)	548	100.0	3,169 (300)	
Time of school enrollment (years)	548	100.0	1.5 (0.1)	
Weight (kg)				
Male	285	52.0	17.1 (1.9)	NS†
Female	263	48.0	16.7 (1.5)	
Height (cm)				
Male	285	52.0	105.0 (0.1)	NS†
Female	263	48.0	104.0 (0.1)	
General BMI				
< Z-score -3	0	0		
-3 < Z-score < -2	13	2.4		
-2 < Z-score < +1	467	85.2		p<0.001‡
+1 < Z-score < +2	45	8.2		
+2 < Z-score < +3	23	4.2		
> Z-score +3	0	0		
Boys' BMI				
< Z-score -3	0	0		
-3 < Z-score < -2	11	4		
-2 < Z-score < +1	247	87		p<0.001‡
+1 < Z-score < +2	16	6		
+2 < Z-score < +3	11	4		
> Z-score +3	0	0		
Girls' BMI				
< Z-score -3	0	0		
- 3 < Z-score < -2	2	0.7		
-2 < Z-score < +1	220	84		p<0.001‡
+1 < Z-score < +2	29	11		
+2 < Z-score < +3	12	4		
> Z-score +3	0	0		
Average number of family members	5.0 ± 0.0			
Mother's level of education (years)			10.0 (0.1)	
≤ 8	213	38.9		
9-12	312	56.9		p<0.001‡
≥ 13	23	4.2		
Father's level of education (years)			9.0 (0.1)	
≤ 8	239	43.7		
9-12	240	43.8		p<0.001‡

(*continue*)

TABLE 1 (Cont.) Characterization of the preschool children studied.

Variables	N	%	Mean (SD)	Statistics
≥ 13	35	6.3		
No information	34	6.2		
Family's monthly income				
< 1	165	30.1		
1-2	327	59.7		p<0.001‡
≥ 3	56	10.2		

*χ^2 test; †: Student's t-test; ‡ Kruskal-Wallis test; 5% significance level. NS: non significant; <Z-score -3: extremely thin; >Z-score -3 and <Z-score -2: thin; >Z-score -2 and <Z-score +1: normal weight; >Z-score +1 and <Z-score +2: risk of excess weight; >Z-score +2 and <Z-score +3: excess weight; >Z-score +3: obesity; BMI: body mass index.

TABLE 2 Socioeconomic variables of the preschoolers' families.

Variables	N	%	Mean (SD)
Rooms per residence			6.0 (0.0)
Type of residence			
Rammed earth	23	4.2	
Bricks/cement	525	95.8	
Electricity			
Yes	548	100.0	
No	–	–	
Public water supply			
Indoors	536	97.8	
Out of the house	12	2.2	
Garbage collection			
Yes	548	100.0	
No	–	–	
Public wastewater			
Sewage	486	88.7	
Septic tank	62	11.3	
Paved street			
Yes	255	46.5	
No	293	53.5	
Child was ill in the last 15 days			
Yes	100	18.2	
No	448	81.9	
Symptoms presented			
Diarrhea	8	8.0	
Coughing	33	33.0	
Fever	59	59.0	
Control of parasitic diseases			
Yes	10	1.8	
No	538	98.2	
Use of medication			
Yes	58	10.6	
No	490	89.4	

TABLE 3 Percentage of food items consumed by preschoolers.

Food item	Consumes	Does not consume
Chocolate milk	91.7	8.3
Rice	100.0	–
Tapioca pancake, couscous	79.5	20.5
Wafers, cakes, sweet pies	94.1	5.9
Coffee	78.9	21.1
Broths and soups	74.2	25.8
Red meat	97.1	2.9
Poultry	87.4	12.6
Breakfast cereals	77.8	22.2
Chocolate and ice cream	77.7	22.3
Processed meats	83.3	16.7
Flours	72.4	27.6
Beans	99.4	0.6
Bovine liver	59.5	40.5
Fried food	87.0	13.0
Fruit	98.5	1.5
Vegetables	75.9	14.1
Yogurt	86.9	13.1
Milk and dairy products	94.1	5.9
Pasta	78.3	21.7
Butter and margarine	95.4	4.6
Giblets	39.2	60.8
Porridge	61.8	38.2
Egg	82.2	17.8
Bread	98.5	1.5
Seafood	73.6	26.4
Formula	65.5	34.5
Ham	68.6	31.4
Soft drinks	90.2	9.8
Artificial fruit juices	82.8	17.2
Natural fruit juices	89.8	10.2
Potatoes	76.1	23.9
Smoothies	76.8	23.2

TABLE 4 Correlation between food consumption and nutritional status of the preschool children.

Consumption of food item	Nutritional status		
	Thin	Normal weight	Risk of/Excess weight
	r^2	r^2	r^2
Rice	0.236	0.657*	0.256
Beans	–	0.721*	0.287
Fruit	–	0.756*	0.143
Bread	–	0.453	0.723*
Red meat	0.218	0.856*	0.212
Butter, margarine	–	0.687*	0.651*
Wafers, cakes, sweet pies	0.298	0.489	0.832*
Milk and dairy products	–	0.721*	0.854*
Chocolate milk	–	0.218	0.722*
Soft drinks	–	0.254	0.698*
Natural fruit juices	–	0.465	0.176
Poultry	–	0.854*	0.356
Fried food	–	0.111	0.602*
Yogurt	–	0.643*	0.231
Vegetables	–	0.643*	0.115
Processed meats	–	0.634*	0.754*
Artificial fruit juices	–	0.287	0.376
Pasta	–	0.367	0.432
Coffee	–	0.898*	0.643*
Smoothies	–	0.549	–

(-) Test not performed due to very low consumption; (*) p<0.05: significant association; weak correlation: $r < 0.3$; moderate correlation: $0.3 \leq r < 0.7$; strong correlation: $r \geq 0.7$. Food groups in which no correlation was found: egg, tapioca pancakes and couscous, breakfast cereals, chocolate and ice cream, potatoes, broths and soups, seafood, flours, ham, formula, porridge, bovine liver, giblets.

group showed that children from families with an income of 2 to 3 minimum wages are twice as likely (OR 2.23, 95CI 1.34-3.72) to be overweight than those belonging to families with incomes of up to one minimum wage.[26]

Therefore, income is one of the factors that could encourage the consumption of healthier foods by increasing access to such, meaning that diet may be a protective factor for overweight. However, the most appropriate choices do not depend exclusively on income but also family eating habits, level of education, and the availability of time for the preparation of food, among others.

Considering monetary income as an indicator of Food and Nutrition Security (FNS), it is assumed that increased income may help to create a favorable environment for food security.[27]

The results of this study show that the excess weight of the children studied is well below the national average (36.4%),[3] as opposed to research where 578 children from public day care centers in the urban region of São Paulo, state of São Paulo, Brazil, were evaluated (intervention study), indicating the prevalence of a 28.9% risk of overweight and obesity. There was no significant difference when comparing different age strata.[28] The research also noted a 20.8% risk of overweight, 5.2% overweight and 12.7% obesity in 403 preschoolers from the private education network in Teresina, state of Piauí, Brazil.[29] Closer results were observed by researchers who assessed 1,435 children from the urban and rural zones of Pernambuco, Brazil, and determined a prevalence of 9.5% overweight and 3.8% obesity.[21]

The National Demography and Health Survey showed a prevalence of 7.8% of some degree of overweight among children aged under 5 years, associating this condition with the early introduction of foods, consumption of processed foods and sedentary lifestyle.[30]

In quilombo communities, a 6.0% prevalence of obesity was found by analyzing 724 children in the preschool age range from quilombo communities.[31] The closest results to the present study were observed in research that indicated an 8.2% prevalence of overweight and 2.5% of obesity.[32] Most studies show that overweight has a higher prevalence than obesity, with a variation in these results with respect to age group and sex.[32,33]

Considering that we studied children from all regions of the city, the results obtained are satisfactory with regard to housing conditions, given that almost the entire sample is served by basic services such as water supply, electricity and garbage collection, but insufficient basic sanitation. It is known that access to such services may influence the child's general health conditions and, therefore, their nutritional status.

In general, certain reasons for the results observed in this study may be proposed, including the improvement of population's living conditions, probably due to certain welfare programs, as well as greater access to healthcare. In addition to these, it is worth mentioning the protective effect due to the presence of these children in the day care centers, where the food provided must meet 30% of the daily nutritional requirements, distributed in at least two meals.[34]

The relationship between attendance at the day care center and the nutritional status of preschool children has been gaining interest in the current scenario, given that the children spend most of their day at such institutions, meaning that day care centers are responsible for providing most daily meals and, consequently, the supply of nutrients.[26] In addition, these institutions no longer have merely a "supporting role" and have assumed an important function in child education, including the promotion of health actions that interfere in the nutritional status of preschool children.

The qualitative analysis of the children's diet does not allow us to infer about quantitative adequacy. However, it does enable an assessment of the diversity of the diet, given that it is known that a diversified diet is associated with greater quantitative adequacy, as it implies the intake of various nutrients.

The assessment of the usual consumption of food allows us to predict the risk that the consumption of certain foods can entail in the development of certain diseases, as it is possible to identify the cumulative effect of several nutrients simultaneously on health.[35]

According to Vilela et al.,[36] food choices are extremely important to proper growth and development because the quality and quantity of food ingested interferes with the supply of nutrients and nutritional status. The food consumption profile of the group under study was characterized by frequent intake of foods considered as traditional, such as "rice and beans," which is typical of the Brazilian culture, as well as other fundamental foods for appropriate child growth and development, such as milk and dairy products and red meat, which are important sources of protein, in addition fruits, which are sources of essential vitamins and minerals.

When analyzing which foods are consumed the most in Brazil, it was noted that those with the greatest frequency of use throughout the national territory and in all levels of family income were rice, beans, coffee, bread and beef, respectively.[37] This data is in concordance with the results of this study with regard to the foods rice, beans, bread and red meat.

Similar results were observed in a study[38] analyzing the dietary intake of public and private schoolchildren in São Luís, Brazil. The foods consumed the most were rice (97.6%), bread (77.6%), beans (61.6%), butter/margarine (61.1%), and beef (59.6%).

Another unfavorable aspect observed in the diet of these children was the large number of children frequently consuming soft drinks to the detriment of the consumption of natural fruit juices and smoothies. Several reasons can be suggested as determinants, including convenience and lower cost. Excessive consumption of soft drinks and industrialized juices, foods with low levels of vitamins and minerals and high levels of additives and sugars, is a concern, given that drinks are capable of increasing the energy content of the diet and encouraging the emergence of obesity and related complications.[39]

The parents' choice of which foods to offer their children may be due to a lack of sufficient knowledge about the most important foods for the nutrition and development of these children and the association between the consumption of certain foods with social status and higher purchasing power because they are constantly exposed by the media.

In general, it could be said that this is not a very diversified diet, which can also be seen in a study that showed the consumption of a diet with little variety, with a high intake of cereals and low consumption of fruits, legumes and vegetables.[31]

Although ours is a low-income population, the vast majority of those responsible for the children reported the frequent consumption of food declared as being high cost. This fact is not in agreement with research that shows a trend of reduced consumption of foods such as meat, milk and dairy products, fruits, vegetables and legumes the lower the family income, due to the high cost of these foods.[40]

Data from the POF 2002-2003 further showed that lower income groups presented purchases of lower cost foods in order to achieve adequate caloric intake, such as oils, flours, cereals, cookies and soft drinks. This associa-

tion is easily justified by the inverse correlation between the cost of food and its energy density.[41]

Research conducted with children using the public health network in Aracaju, Brazil, indicated high food consumption of cereals, meat, sugars, oils and fats and low consumption of fruits and vegetables.[42] In the group under study it was also possible to note that the milk and dairy products food group was the one with the greatest share in the diet of this population, probably for dealing with children aged 6-35 months, a period where parents prioritize a milk-based diet, in the form of porridge, mash and purees.[42]

Considering the correlation between food consumption and nutritional status, a similar result was observed, especially the excessive consumption of soft drinks and artificial juices as risk factors for overweight in preschool age.[18]

On the other hand, studies show that the frequent consumption (three times per week) of fruits and vegetables showed a protective effect in the development of overweight and obesity, considering children between 5 and 9 years.[43,44]

For some authors,[36,45] the study of the diet with various combinations of food rather than the consumption of individual food items or the intake of nutrients may be of greater interest, considering that foods are not consumed in isolation and reflect each individual's choice for a particular lifestyle.

Conclusion

The results of our study were positive regarding the prevalence of overweight and obesity among the group of preschoolers, showing prevalence well below the national average. In relation to food intake, it should be noted that the presence of some foods like cookies, cakes, pies and chocolate milk is more frequent than ideal, and these foods were declared by a large proportion of children. It is noteworthy that these are foods high in sugars, which undermines the concept of healthy eating and may favor the emergence of overweight and obesity.

Given these results, food and nutrition policies that encourage the consumption of healthy foods and maintain the consumption of basic traditional foods such as rice and beans are necessary, while at the same time encouraging a reduction in the consumption of processed foods that are high in sodium, saturated fat and simple sugars.

Acknowledgments

Thanks to the Coordination of Improvement of Higher Level Personnel (CAPES) for the master's degree scholarship awarded, the Municipal Department of Education, and the principals, professors, parents/guardians and children for their participation.

Resumo

Excesso de peso e obesidade em pré-escolares: prevalência e relação com consumo alimentar

Objetivo: Determinar a prevalência de excesso de peso e obesidade em pré-escolares de instituições públicas de ensino e sua relação com consumo alimentar.
Método: Estudo transversal com crianças de 2 a 5 anos, de ambos os sexos, atendidas em creches municipais. Coletaram-se dados socioeconômicos, demográficos e antropométricos, para cálculo do índice de massa corpórea (IMC) por idade. Os dados sobre consumo alimentar foram avaliados por meio de Questionário de Frequência Alimentar. Utilizaram-se os testes do χ^2, Kruskal-Wallis, t de Student e correlação de Pearson, com nível de significância de 5%.
Resultados: Das 548 crianças, 52% eram do sexo masculino, com média de idade de 4,2 anos. A maioria das famílias apresentou renda entre 1 e 2 salários mínimos (59,7%) e escolaridade materna de 10 anos. Os parâmetros antropométricos não apresentaram diferença significativa entre os sexos. Segundo o IMC/I, verificou-se que a maioria das crianças estava eutrófica (85,2%); 8,2%, com risco de excesso de peso; 4,2%, com excesso de peso. Os alimentos mais consumidos foram: arroz (100%), feijão (99,4%), pães (98,5%), frutas (98,5%), carne vermelha (97,1%), manteiga e margarina (95,4%), bolachas, bolos, tortas doces (94,1%), leite e derivados (94,1%), achocolatado (91,7%) e refrigerantes (90,2%). Os alimentos consumidos que apresentaram forte correlação ($r > 0,7$) com o risco/excesso de peso foram: pães; bolachas, bolos, tortas doces; leite e derivados; achocolatados e embutidos.
Conclusão: Observaram-se baixa prevalência de excesso de peso e ausência de obesidade entre o público pesquisado. O risco de excesso de peso foi maior entre as meninas. O estudo mostrou desvios no consumo alimentar.

Palavras-chave: excesso de peso, obesidade, pré-escolar, consumo alimentar.

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Qualitative and quantitative ultrasound assessment of gastric content

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Summary

Objective: Pulmonary aspiration of the gastric contents is one of the most feared complications in anesthesia. Its prevention depends on preoperative fasting as well as identification of risky patients. A reliable diagnostic tool to assess gastric volume is currently lacking. The aim of this study performed on volunteers was to evaluate the feasibility of ultrasonography to identify qualitative and quantitative gastric content.

Method: A standardized gastric scanning protocol was applied on 67 healthy volunteers to assess the gastric antrum in four different situations: fasting, after ingesting clear fluid, milk and a solid meal. A qualitative and quantitative assessment of the gastric content in the antrum was performed by a blinded sonographer. The antrum was considered either as empty, or containing clear or thick fluid, or solids. Total gastric volume was predicted based on a cross-sectional area of the antrum. A p-value less than 0.05 was considered statistically significant.

Results: For each type of gastric content, the sonographic characteristics of the antrum and its content were described and illustrated. Sonographic qualitative assessment allowed to distinguish between an empty stomach and one with different kinds of meal. The predicted gastric volume was significantly larger after the consumption of any food source compared to fasting.

Conclusion: Bedside sonography can determine the nature of gastric content. It is also possible to estimate the difference between an empty gastric antrum and one that has some food in it. Such information may be useful to estimate the risk of aspiration, particularly in situations when prandial status is unknown or uncertain.

Keywords: gastric content, antral area, pulmonary aspiration, preoperative fasting, ultrasonography.

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Article received: 5/24/2016
Accepted for publication: 9/3/2016

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Funding: Fundação de Amparo à Pesquisa de Minas Gerais (Fapemig) and Fundação de Ensino e Pesquisa de Uberaba (Funepu)

http://dx.doi.org/10.1590/1806-9282.63.02.134

Introduction

Since Mendelson's description of gastric aspiration syndrome[1] in anesthetized parturient women in 1946, gastric content has become a constant concern among anesthesiologists, leading to the development of preoperative fasting guidelines.[2,3] However, certain clinical conditions may predispose elective patients to present significant gastric content at the time of induction of anesthesia, even if the fasting time is appropriate.[4,5] This problem is even greater when dealing with emergency surgeries. In this circumstance, gastrointestinal motility may be influenced by stress, the presence of pain and anxiety and the use of opioids, making it difficult to predict the gastric condition. Patients who have a "full stomach" are at risk of aspiration during sedation or general anesthesia due to reduction of the tonus of the lower esophageal sphincter and also protective airway reflexes.[6,7] Certain factors have been associated with the severity of the evolution of patients suffering from pulmonary aspiration, including the volume, nature and the pH of the aspirated material.[8-11]

The preoperative assessment of the risk of pulmonary aspiration is essentially based on the patient's history, and clinical management typically follows the fasting recommendations of the current guidelines.[2,3]

Unfortunately, a final assessment of the nature and volume of the gastric content at bedside at any time is not easy, thus being inaccessible for anesthesiologists. Scintigraphy has been considered the gold standard for this assessment for many years.[12,13] However, due to the cost, radiation exposure and the need for specific equipment, this technique has only been useful for research proposals. Gastric ultrasonography (USG) is the first non-invasive imaging examination that has been validated for this purpose, because it can provide information about the nature and volume of the gastric content at bedside.[14-17] These facts have contributed to its use as a replacement technique, given that it is inexpensive and can be performed at bedside. USG versatility has enabled its use during the perioperative period for the assessment of the gastric content, a parameter of great importance especially in emergency situations, with the objective of providing more rational management and reducing the risk of aspiration.

Recent studies have shown sufficient evidence of its accuracy and reproducibility.[14-17] Obviously, ultrasound examination does not provide a complete assessment of gastric function and status (e.g. pH), but it can provide important and useful information such as the volume and nature of the content (clear liquid, solid or none).[14-19]

Considering that USG is an examination rarely used for this purpose in our country, the objectives of this study were: 1) to describe the appearance of the stomach while empty and after ingestion of fixed volumes of different types of food; 2) to describe the interpretation of the radiological image undertaken by the examiner in relation to the type of food ingested; and 3) to evaluate the usefulness of the gastric USG in quantifying the gastric content following the ingestion of different foods and thereby use it as a tool for assessing the risk of gastric aspiration.

Method

After approval by the Research Ethics Committee (CEP) of the Federal University of the Triângulo Mineiro (UFTM) under number 1.448.546 and obtaining the informed consent of the participants, this cross-sectional and prospective study was conducted on 30 healthy volunteers. The inclusion criteria were: being aged between 20 and 60 years, physical status classification according to the American Society of Anesthesiologists (ASA) I or II, body mass index (BMI) of less than 30 kg/m^2, and the ability to understand the study protocol and informed consent. The exclusion criteria were considered as any condition that could interfere with the gastric emptying time, such as pregnancy, diabetes, or the presence of diseases of the gastrointestinal tract.

The volunteers were told to undergo a minimum nighttime fasting period of 8 hours, after which they would be examined randomly in several different situations, designated as the following groups: a) fasting (fasting group); b) 5 minutes after ingestion of 250 mL of clear fluid (isotonic solution) (isotonic group); c) 10 minutes after ingestion of 250 mL of non-clear liquid (milk group) (group L); or d) 10 minutes after ingestion of a solid meal (ham and cheese sandwich) (sandwich group). We established the volume of 250 mL of isotonic liquid and milk because this amount is greater than the upper limit of gastric volume of normal baseline fasting.[20]

Ultrasound assessment of the gastric content was conducted by an experienced professional at the Radiology Service of UFTM who was unaware of the quality or quantity of the ingested material. The tests were carried out using a technique described beforehand,[14,15] using a convex probe (2-5 MHz). The volunteers were examined in supine position, followed by right lateral decubitus position (RLDP). The transducer was placed on the sagittal plane in the epigastric region and the antrum and the gastric body were then scanned by moving the transducer from right to left, with the purpose of obtaining a general qualitative view of the cavity and the gastric content. A better view of the antrum is obtained on the parasagittal plane just to the right of the midline, with the left lobe of the liver anteriorly and the pancreas posteriorly as a reference point. The inferior vena cava is situated behind the pancreas. The gastric antral wall is characterized by multiple layers, and its visibility was assessed in a binary way (visible or not) in both positions, supine and RLDP. At visualization of the antrum, the stomach was considered empty if the anterior and posterior walls appeared juxtaposed. The antrum was regarded as containing liquid if it showed distended walls as well as endocavity with hypoechoic or anechoic content.[15,20,21] The images obtained after intake of milk and solid foods differ, depending on the time between ingestion and the ultrasound examination. The presence of milk, as well as thick fluids, increases the echogenicity.[20] After a solid meal there is a substantial amount of air mixed with the *bolus* created by the processes of chewing and swallowing. The mixture of air and solid creates the image of multiple artifacts in the anterior gastric wall, which typically blurs the posterior wall of the antrum.[15,20] Due to the presence

of air or gas bubbles an image with multiple points of echoes appears. After some time, the air moves from the stomach and the solid content can be better viewed as a mixture of echogenicity similar to "frosted glass." Peristaltic contractions are often observed in both the antrum and pylorus, particularly when there is content present. All images were obtained between peristaltic contractions.

Both qualitative and quantitative information of the gastric content were obtained. The qualitative assessment of the stomach was described initially, after identification of the gastric antrum. Four ultrasound images were considered, given that the examiner conducted the tests without knowing the nature of the ingested material. Therefore, the result described was based on the echogenicity of the image obtained. The examiner described the stomach in the following ways: 1) empty; 2) presence of liquid content when the image was hypoechoic; 3) solid content if hyperechoic and homogeneous, which corresponds to the ingestion of milk; and 4) gaseous image, due to the presence of hyperechoic points, which denotes the presence of gas and hence refers to the ingestion of solids (sandwich).

We used the measurement of the antral cross-sectional area (CSA) for a quantitative analysis, utilizing the technique described by Bolondi,[22] and later by Perlas et al.[14-16] evaluating the outer wall of the stomach. This was done in RLDP using two perpendicular diameters of the antrum, from serosa to serosa, the longitudinal or craniocaudal (CC) and the anteroposterior (AP) diameter, using the ellipse formula developed by Bolondi et al.,[22] where CSA = (CC x AP x π)/4. Where the value of π = 3.14.

After calculating the CSA, the total volume of the stomach ("anticipated volume") was estimated in each volunteer using a mathematical model previously tested and validated by other authors[23] where:

$$\text{Stomach volume (mL)} = 27 + 14.6 \times \text{CSA (cm}^2\text{)} - 1.28 \times \text{age (in years)}$$

The ratio between the predicted volume and the weight of the volunteers was subsequently calculated by simple division.

To demonstrate the results a descriptive analysis of the demographic data (age, weight, height, and BMI), gender and ASA classification were used. The data were summarized using the mean and standard deviation.

The assessment of the different groups by the examiner was undertaken solely with the analysis and interpretation of the image in relation to the food ingested, with the result either right or wrong. The statistical analysis was conducted using Chi-square (χ^2) test with a significance level of 5%.

To compare the quantitative assessment of the gastric variables of the antral area, the predicted gastric volume and volume over the weight of the volunteers, we used the ANOVA test, with a significance level of 5%.

Results

Sixty-seven stomach ultrasound tests were performed on 30 volunteers. There were 19 fasting examinations and 16 examinations after the intake of each type of food: isotonic solution, milk and sandwich. Not all volunteers did the tests in the four situations. In all tests the stomach was located successfully. The data relating to the volunteers can be seen in Table 1.

Fourteen of the 19 tests on fasting volunteers (73.68%) were considered empty by the examiner. In this case, the antrum appears flat with the anterior and posterior walls juxtaposed. On the sagittal plane, it is round to oval and has been compared to a "target" or an image of a "bull's eye" (Figure 1A). On an axial plane, the empty antrum has the appearance of a "glove finger."[14,15,20,21,24] The other four (26.31%) volunteers presented images corresponding to the presence of liquid content in the stomach but with an estimated volume of less than 1.5 mL/kg.

The gastric content of the 16 volunteers who ingested isotonic solution was considered liquid in 13 (81.25%) volunteers and solid in three (18.72%) of them. With the presence of liquid in the stomach, the antrum appears with a distended rounded shape, fine walls and hypoechoic content (Figure 1B). Immediately after the intake of fluids, multiple gas bubbles may appear as hyperechoic points within the hypoechoic fluid, giving the appearance of a "starry night" of varying intensity. This appearance may lead to a different interpretation of the gastric content, as occurred in the three cases that were considered solid.

After the ingestion of 250 mL of milk, the examiner considered the gastric content as solid in 13 (81.25%) volunteers, as liquid in two (12.50%), and empty in one (6.25%). With the intake of milk, the gastric antrum appears round and distended. Its contents, however, differ substantially from clear liquid, appearing with increased echogenicity (Figure 1C). Hypoechoic content with multiple hyperechoic points changed the interpretation in two cases, which were reported to be liquid content, and a case where the antrum appeared empty, with a gastric volume calculated as only 15 mL.

After ingestion of solid content, the examiner found gaseous content in 15 (93.75%) volunteers and solid content in one (6.25%). The ingestion of solid food leads to the ap-

pearance of an image that resembles "frosted glass" (Figure 1D). This is due to the mixture of air with food during the processes of chewing and swallowing. In some circumstances this may limit the view of some of the walls of the stomach, and make it difficult to assess the volume of the organ. The assessment of one case as solid was caused by an absence of air. The description of gaseous content for the ingestion of sandwiches was considered correct since the analysis was only undertaken on the image, ignoring the ingested material.

When analyzing the examiner's ability to discover the nature of the material present in the stomach there was no difference among the four groups ($p>0.05$).

The result of the quantitative assessment of the gastric content, after eating different foods, can be seen in Table 1. There was a significant difference between the data obtained from the fasting volunteers and those who ingested isotonic drinks, milk or a sandwich ($p<0.05$). The gastric volumes were significantly greater with the intake of any of these substances and exceed the limit considered safe for the risk of gastric aspiration of 1.5 mL/kg (Figure 2).

Discussion

The results show that the gastric antrum has a different appearance on ultrasound in the various situations studied, namely after fasting (while empty), after ingestion of clear liquid, milk and solid food. In addition, the gastric antrum expands from the baseline (empty state), with the entry of the volumes ingested, allowing it to be considered as "full," and thus differing from the empty state. Furthermore, based on the image of the antrum it was possible to calculate the cross-sectional area, allowing us to determine the total gastric volume, which proved to be compatible with the amount of food ingested.

One of the main complications faced by anesthesiologists in elective procedures and especially in emergencies, involving instrumentation of the upper airways, is pulmonary aspiration. In the United States, there are reports of an incidence of aspiration of 1:14,500 patients undergoing elective surgery, with a significant increase during tracheal intubation in emergency situations.[24] The mortality rate is around 30 to 70%, an event that is directly proportional to the volume, nature (liquid versus particulate or solid matter), and acidity of the aspirated material.[8-10] Severe pulmonary aspiration requires ventilatory support in more than a third of patients, reaching a mortality rate of 5%, representing more than 9% of all deaths related to anesthesia.[23,25-27] Current strategies to prevent aspiration depend mainly on the recommended fasting periods.[2,3] However, various medical conditions may predispose patients to present a high gastric content during anesthetic induction, regardless of the fasting time.[5,28] This occurs, for example, in patients with slow gastric emptying such as patients with diabetes mellitus.[29] On the other hand, patients who undergo emergency surgery often have significant gastric content, even if they are within the fasting time considered as being safe.

After the initial descriptions of gastric aspiration leading to severe pneumonias, a large number of studies have aimed to determine the minimum gastric volume responsible for pulmonary parenchymal lesions. Although there is no strict "threshold volume" above which there is an increased risk of aspiration, gastric fluid volumes up to 1.5 mL/kg (around 100 mL for an average adult) are common in fasting patients and are considered safe.[30-33] In our study, the ultrasound assessment of the gastric volume of volunteers with a fasting period of over 8 hours led the exam-

TABLE 1 Volunteer data and results of the USG assessment according to the group.

	Fasting group	Isotonic group	Milk group	Sandwich group
Age (years)	33.10 ± 9.94	30.50 ± 8.21	33.12 ± 8.37	31.56 ± 8.51
Weight (kg)	68.42 ± 11.73	68.18 ± 12.00	70.86 ± 11.32	70.16 ± 11.68
BMI (kg/m²)	24.02 ± 2.76	23.88 ± 2.66	24.22 ± 2.81	24.39 ± 3.55
ASA	ASA 1: 16	ASA 1: 14	ASA 1: 11	ASA 1: 15
	ASA 2: 3	ASA 2: 2	ASA 2: 5	ASA 2: 1
Gender	Male: 7	Male: 6	Male: 5	Male: 5
	Female: 12	Female: 10	Female: 11	Female: 11
Area (cm²)	4.89 ± 1.81*	15.28 ± 5.32	16.41 ± 6.71	10.89 ± 3.62
Volume (mL)	56.08 ± 25.96*	211.19 ± 78.26	224.21 ± 98.12	145.62 ± 56.02
Volume/Weight (mL/kg)	0.82 ± 0.37*	3.16 ± 1.33	3.28 ± 1.81	2.08 ± 0.73

There was no difference in the demographic data of the volunteers in the four situations studied ($p>0.05$). There was a significant difference between the fasting group and the remaining regarding data obtained on USG (*$p<0.05$).
ASA: physical status classification according to the American Society of Anesthesiologists; BMI: body mass index; USG: ultrasonography.

iner to consider the stomach as being empty in 73.68% of the reports, with the presence of liquid content observed in 26.3%. This is certainly a result of normal gastric secretion because the volumes were below the limits considered at risk for aspiration (less than 1.5 mL/kg). Meanwhile, for the other groups that ingested isotonic solution, milk or a solid meal, the antrum was only considered empty in one case (2%) and presented an estimated volume of 0.4 mL/kg, although that was a volunteer who ingested milk. All of the others presented increased content within the stomach and thus were considered as a "full stomach." The presence of particulate matter was found in volunteers who ingested milk and solid meals.

In anesthesiology and emergency medicine, there is a great interest in assessing the state of gastric "fullness" at the bedside in order to assess the risk of pulmonary aspiration. Clinical decisions relating to the time of surgery and the choice of airway approach are based on the assumption that the patient has an "empty stomach" or "full stomach," according to the time elapsed since the last meal. However, the gastric emptying time varies significantly depending on pre-existing conditions, and fasting guidelines do not always guarantee an "empty stomach" in patients with gastric motility changes of any etiology.[29] Furthermore, patients who undergo emergency surgeries normally have not fasted, or they may have a significant gastric content despite long periods without eating. Therefore, when the gastric "status" is unclear or unknown, an ultrasound examination performed at bedside can be very useful in assessing the risk of aspiration. In these cases, if the examination confirms the presence of a thick liquid or solid content, this is clearly a situation of high risk for aspiration, regardless of the exact volume. On the other hand, if the ultrasound

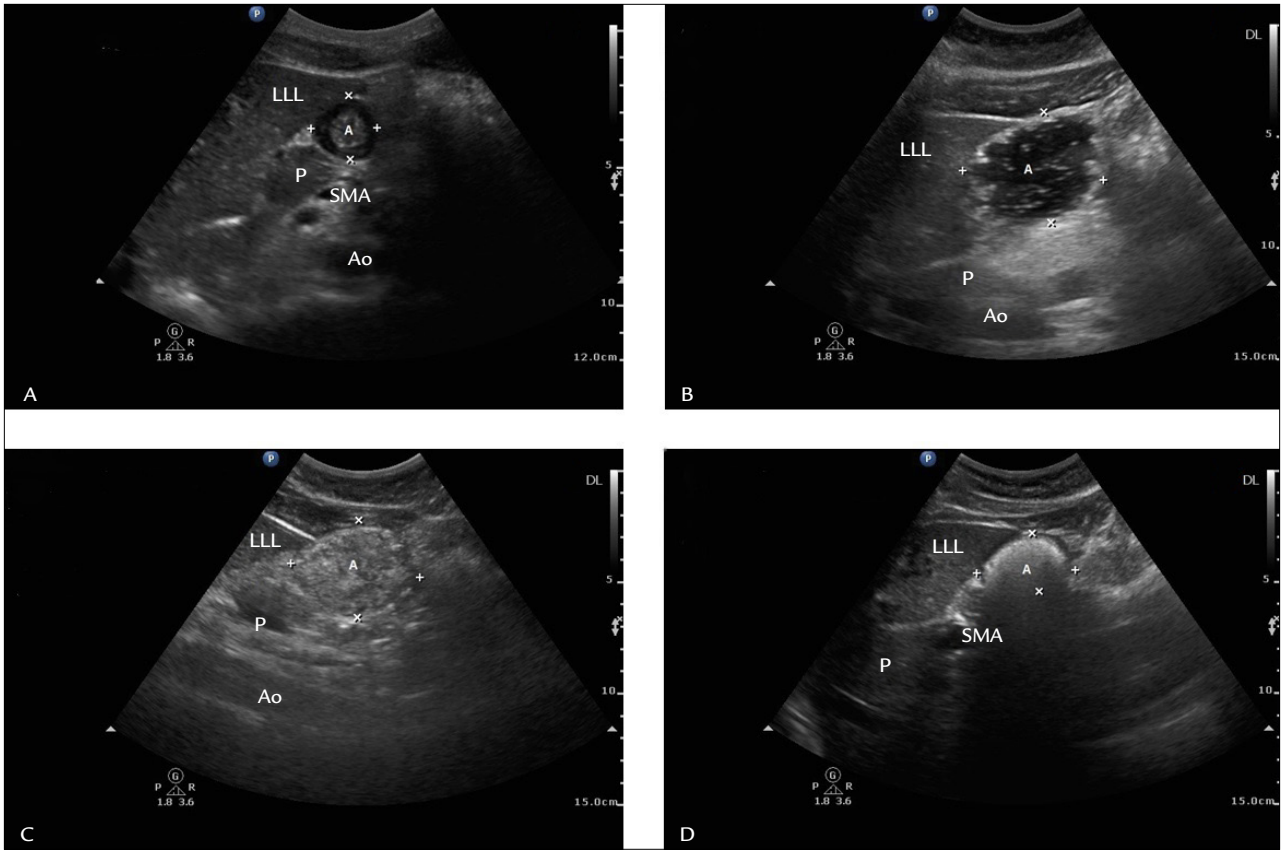

FIGURE 1 A. Ultrasonography image of the gastric antrum with an empty stomach. The antrum appears small and empty, with a "bull's eye" appearance. B. Ultrasonography image of the gastric antrum after ingestion of a clear fluid. Note that the antrum appears distended with hypoechoic or anechoic content. Small gas bubbles giving the appearance of a "starry night." C. Ultrasonography image of the gastric antrum after ingestion of milk. The antrum appears round and distended. There is increased echogenicity, with an image that resembles coagulated content. D. Ultrasonography image of the gastric antrum 10 minutes after ingestion of solid food (a sandwich). Image that resembles "frosted glass."
A: gastric antrum; LLL: left lobe of the liver; P: pancreas; SMA: superior mesenteric artery; Ao: Aorta.

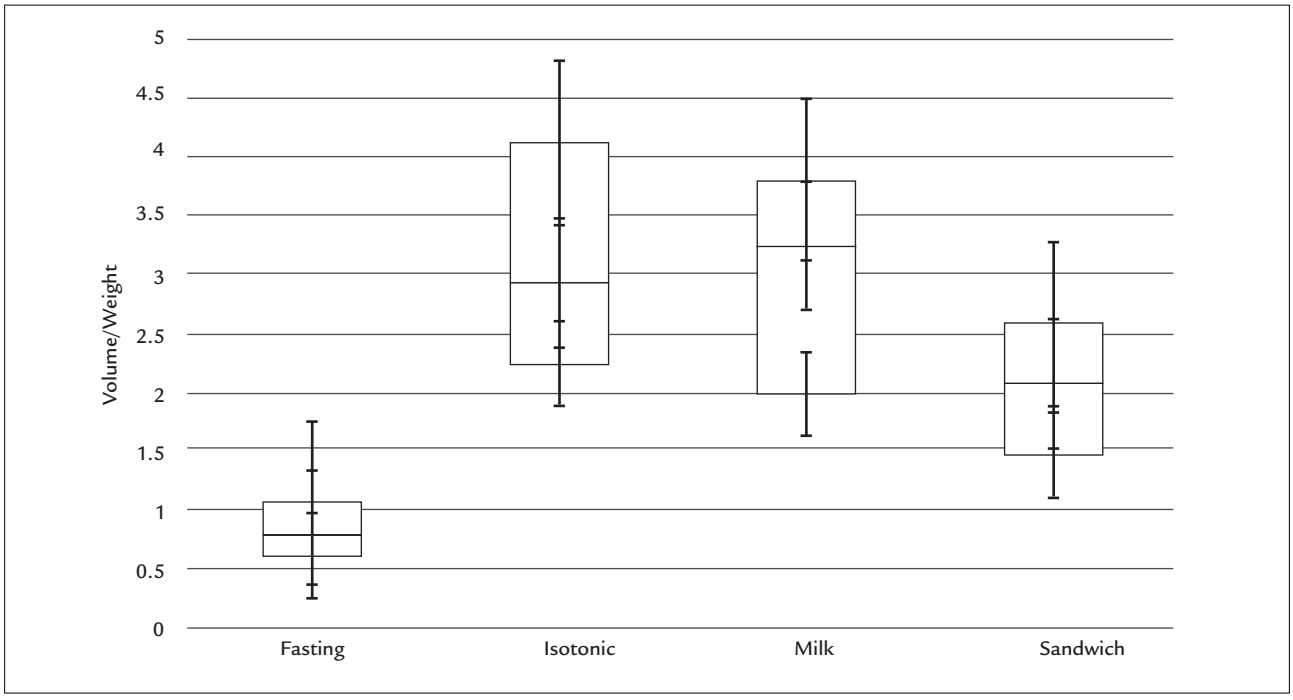

FIGURE 2 The volume/weight ratio was significantly lower in the fasting group compared to the three remaining groups ($p<0.05$), which exceed the limit considered safe for risk for pulmonary aspiration of 1.5 mL/kg.

examination reveals an empty stomach, this is clearly a situation of low risk for aspiration. The presence of an empty stomach is a qualitative measurement, and does not require an assessment of the volume. However, if qualitative analysis identifies the presence of a liquid content, the quantitative assessment of the volume may help differentiate between a state of low volume (similar to the physiological baseline gastric content) and a state of greater volume and, therefore, greater risk.[15,21]

In recent years the use of USG by anesthesiologists has been a perioperative resource that has improved several approaches, such as peripheral and spinal nerve locks, and facilitated venous and arterial punctures, cardiac procedures and many others. A growing body of evidence shows the benefits of the change in practice.[34] The recent interest in the assessment of the stomach in order to evaluate the volume qualitatively and quantitatively before anesthesia plays a very important role in guiding decision-making. Determining the presence of an increased volume in the stomach helps to establish the risk of perioperative aspiration and thus serves as a guide in choosing the best preventive strategy. Up to now, the clinical applications of USG have been limited, although for more than two decades gastroenterologists have used this technique to access gastric motility and emptying[35,36] and for the diagnosis of lesions of the stomach wall, such as cancer.[37-39] Some authors have used USG to differentiate the nature of the gastric content.[40] Bolondi[22] undertook the imaging of the gastric antrum in a transversal view and calculated its area. Based on this study, sequential measurements of the area of the antrum after the ingestion of standard meals were used to assess the gastric emptying time, with a good correlation with the scintigraphy.[29,41,42] An observational study of 183 patients for elective or emergency surgery measured the gastric antrum area immediately before the induction of anesthesia. After tracheal intubation, a multi-hole 18 F probe was inserted into the stomach and its contents aspirated. There was a significantly positive correlation between the area of the antrum and the gastric volume aspirated.[17]

The USG technique is based on the insonation of several projections of the stomach, which facilitates the calculation of the transversal area of the gastric antrum and, as such, makes it possible to obtain different measurements that allow the correct assessment and characteristics of the gastric content.[5] Several studies suggest that the gastric antrum is the region of the stomach that is the most amenable to ultrasound examination,[14,18,20] and is identified in 98 to 100% of cases.[15,17,21] By calculating the cross-sectional area of the antrum based on USG image, several mathematical models have been developed to determine the gastric volume.[14-17] Furthermore, this

method of assessing the gastric volume has proven to be reproducible and has little variability either when assessed by a single or multiple evaluators.[43] The results were also shown to be equivalent with another assessment method that takes a dimensional measurement using the device's software, and this is not based on the assumption that the gastric antrum is a perfect ellipse.[14,22,36,43] The method can predict volumes of 0 to 500 mL and is applicable to adult patients with a BMI less than 40 kg/cm^2. It has sensitivity and specificity of 100%, and is considered the gold standard for non-invasive assessment of the stomach. The margin of error in the measurements is only ± 6 mL.[16]

It may currently be premature to anticipate how this new technology could affect the daily practice of anesthesiologists, although we believe it to be a promising clinical skill to be developed. More research needs to be conducted in relation to the sensitivity and specificity of the method, although it has been proven as a valid technique.[16,17]

There are several limitations in this study. First, it was conducted on healthy adult volunteers. As such, the results should not be extrapolated to other patient populations, such as children, the obese, or patients with specific diseases. Secondly, limited quantities of liquids and solid meals were ingested, meaning that larger volumes could render different images than the ones obtained. Finally, the tests were performed by a single examiner, a radiologist, using a high-definition device, making it difficult to extrapolate USG as a tool for use in anesthesiology at any time.

Conclusion

Our data suggest that USG can offer qualitative and quantitative information of the gastric content that can help in assessing the stomach for the risk for pulmonary aspiration.

Conflict of interest

The authors declare no conflict of interest.

Resumo

Avaliação qualitativa e quantitativa do conteúdo gástrico através da ultrassonografia

Objetivo: A aspiração pulmonar do conteúdo gástrico é uma das complicações mais temidas em anestesia. A sua prevenção depende do jejum pré-operatório e da identificação dos pacientes de risco. Não há um método diagnóstico que possa acessar o conteúdo gástrico a qualquer momento. O objetivo deste estudo realizado em voluntários foi fazer uma avaliação qualitativa e quantitativa do conteúdo gástrico utilizando a ultrassonografia.

Método: O estudo foi realizado em 67 voluntários utilizando uma técnica já descrita de avaliação do antro gástrico, em quatro diferentes situações: jejum, após a ingestão de líquido claro, leite ou refeição sólida. Foi feita uma avaliação qualitativa e quantitativa do conteúdo gástrico por um radiologista que desconhecia o estado gástrico do voluntário. O antro foi considerado vazio, contendo líquido claro ou espesso, ou sólido. O volume total do estômago foi calculado com base na área seccional do antro. Um valor de $p<0,05$ foi considerado estatisticamente significativo.

Resultados: Para cada tipo de conteúdo gástrico, as características ultrassonográficas do antro e de seu conteúdo foram descritas e ilustradas. A avaliação qualitativa pode distinguir um estômago vazio de outros com diferentes conteúdos. O volume gástrico calculado foi significativamente maior após a ingestão de qualquer alimento em comparação com o jejum.

Conclusão: A ultrassonografia à beira do leito pode determinar a natureza do conteúdo gástrico. Também foi possível diferenciar um antro vazio daquele com algum volume. Essas informações podem ser úteis na determinação do risco de aspiração gástrica, principalmente se a condição gástrica é desconhecida ou incerta.

Palavras-chave: conteúdo gástrico, área antral, aspiração pulmonar, jejum pré-operatório, ultrassonografia.

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Influence of morbid obesity on physical capacity, knee-related symptoms and overall quality of life: A cross-sectional study

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Summary

Objective: To evaluate the impact of morbid obesity on physical capacity, joint-related symptoms, and on the overall quality of life.
Method: Cross-sectional study carried out at a university hospital, enrolling 39 individuals admitted to a bariatric surgery service. Physical capacity was assessed by Six-Minute Walk Test (SMWT) and the Borg rating of perceived exertion (RPE). Knee-related symptoms were evaluated by Knee Injury and Osteoarthritis Outcome Score (KOOS) and the Lysholm Score. Quality of life was evaluated by Short Form 36 Health Questionnaire (SF-36).
Results: On SMWT, the mean distance walked was 374.1±107.5 m. The mean Borg score was 12.9±2.4. KOOS questionnaire found the following scores: pain (64.3±24), other symptoms (67.2±25.5), function in daily living (60.4±26.8), function in sport and recreation (28.5±32.2), knee-related quality of life (35.9±33.5), mean Lysholm scale score (55.3±25.4). SF-36 provided the following scores: physical functioning (41±27.4), physical role functioning (34.6±39.2), bodily pain (45.7±23.6), general health perceptions (63.1±26.2), vitality (53.5±12.1), social role functioning (52.6±29.3), emotional role functioning (41±44.9), mental health (55±27.7).
Conclusion: Obesity led to significant loss of physical capacity, gait impairment, knee-related symptoms, and a negative impact on the overall quality of life.

Keywords: obesity, quality of life, knee, joint diseases, arthralgia.

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Article received: 6/14/2016
Accepted for publication: 6/26/2016

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http://dx.doi.org/10.1590/1806-9282.63.02.142

Introduction

Obesity is a medical disorder characterized by an abnormal accumulation of excess body fat, which is associated with adverse health effects. It has become a worldwide public health concern and, according to World Health Organization (WHO) reports, at least 2.8 million people each year die as a result of being overweight or obese.[1]

Along with diet, behavioral, and drug treatment strategies, physical activity plays a key role in the therapy of severe obesity. Most of the currently promoted exercise regimens include walking. However, recent evidence has shown a significant impairment in physical capacity associated with obesity, such as gait disturbances, posture deficits, and greater risk of falling.[2-6] Furthermore, obesity presents a close relationship with chronic degenerative osteoarticular disease, which contributes even more to physical impairment and disability.[4-6]

There are a few studies evaluating functional abilities, physical capacity, and joint-related symptoms in morbidly obese individuals, as well as their impact on the overall quality of life.

Objective

This study sought to study the influence of morbid obesity on physical capacity, knee-related symptoms, as well as quality of life, in patients eligible for bariatric surgery.

Method

This is a cross-sectional study carried out at a university hospital, enrolling 39 individuals admitted to a bariatric surgery service from May to December, 2011.

Inclusion criteria were: body mass index (BMI) ≥ 40 kg/m^2; age between 18 and 65 years old; capacity to understand the study design and provide informed consent.

Exclusion criteria were: any physical disability not directly related with obesity; refusal to take part in the study; systemic diseases which could prevent the individual to perform the physical tests.

The variables evaluated were: age, gender, ethnic group, weight, BMI, age at onset of obesity, clinical condition, physical activity level, objective and subjective evaluation scores regarding knee-related symptoms, and quality of life.

Clinical condition was assessed based on heart rate, systolic and diastolic blood pressures, and respiratory rate. Patients were evaluated at rest and after physical exercise. Heart and respiratory rates were directly calculated by the researchers, and blood pressure was measured using an analogue calibrated sphygmomanometer.

To evaluate physical activity, the participants underwent a Six-Minute Walk Test (SMWT) and the Borg rating of perceived exertion. The Knee Injury and Osteoarthritis Outcome Score (KOOS) and Lysholm Score were used to assess symptoms related with the knees. The Short Form 36 Health Survey (SF-36) was used to assess quality of life. All of these scores and questionnaires have been previously validated.[7-11]

This study was assessed and approved by the local Research Ethics Board. All individuals provided informed consent. All of the forms and questionnaires were personally applied by the researchers.

Six-Minute Walk Test (SMWT)

The SMWT is a practical simple test that requires a 100-ft hallway but no exercise equipment or advanced training for technicians. It evaluates the global and integrated responses of all the systems involved during exercise, including the pulmonary and cardiovascular systems, systemic circulation, peripheral circulation, blood, neuromuscular units, and muscle metabolism. Most patients do not achieve maximal exercise capacity during the SMWT; instead, they choose their own intensity of exercise and are allowed to stop and rest during the test. However, because most activities of daily living are performed at submaximal levels of exertion, the SMWT may better reflect the functional exercise level for daily physical activities.[12]

Scale of Perceived Exertion (Borg scale)

This is a psychophysiological scale that measures feelings of effort, strain, discomfort, and/or fatigue experienced during both aerobic and resistance training. The rating of perceived exertion (RPE) is often measured using a 15 category scale that was developed by Swedish psychologist Gunnar Borg. It is a numerical scale that ranges from 6 to 20, where 6 means "no exertion at all" and 20 means "maximal exertion."[8,13]

Knee Injury and Osteoarthritis Outcome Score (KOOS)

The KOOS is a self-administered questionnaire that includes five outcomes: pain, symptoms, activities of daily living, sport and recreation function, and knee-related quality of life. It was originally developed to assess short- and long-term patient-relevant outcomes following knee injury and arthritis. A 100-point score indicates absence of knee-related symptoms, while a 0-point score reveals extreme knee-related symptoms.[14]

Lysholm Score

The Lysholm knee scale is a condition-specific outcome measure that was originally designed to assess ligament injuries of the knee.[15] It encompasses eight questions, whose ultimate outcome is expressed nominally, that is "excellent" from 95 to 100 points; "good" from 84 to 94; "fair" from 65 to 83; and "poor" when below 65.[16]

Short Form 36 Health Survey (SF-36)

The SF-36 is a 36-item patient-reported survey of patient health. The SF-36 consists of eight scaled scores, which are the weighted sums of the questions in their section. Each scale is directly transformed into a 0-100 scale on the assumption that each question carries equal weight. A score of zero is equivalent to maximum disability and a score of 100 is equivalent to no disability. The eight sections are: vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, and mental health.[17]

Statistical analysis

For comparison of categorical variables, we used Chi-square and Fischer's tests. To compare continuous measures, the Mann-Whitney test was used. The level of significance adopted was 5% ($p<0.05$). The statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software, version 16.0.

Results

Of 39 individuals, 84.6% were female. Mean age was 42.4±10.8 years old. Regarding ethnic groups, 64.1% were White, 10.3% were Black, and 25.6% were mixed. Mean weight was 126.2±24.6 kg; mean BMI was 49.4±6.8 kg/m^2. Table 1 summarizes the demographic and anthropometric characterizations of the population study.

TABLE 1 Demographic and anthropometric characteristics of the studied population.

Age (years)	42.4±10.8
Gender	Female: 33 (84.6%)
	Male: 6 (13.4%)
Ethnic group	Whites: 25 (64.1%)
	Blacks: 4 (10.3%)
	Mixed: 10 (25.6%)
Weight (kg)	126.2±24.6
BMI (kg/m^2)	49.4±6.8

BMI: body mass index.

Mean heart rate at rest was 73.1±14.3 bpm; after SMWT, it was 113.5±20.3 bpm (p<0.0001). Mean respiratory at rest was 19±4.9 breaths per minute; after SMWT, it was 24.5±8.2 breaths per minute (p=0.01). Mean systolic pressure at rest was 136.7±20.9 mmHg; after SMWT, it was 157±22.3 mmHg (p<0.0001). Mean diastolic pressure at rest was 95±16.7 mmHg; after SMWT, it was 91.4±15.7 mmHg (p=0.19). The SMWT yielded the following results: mean speed was 63.6±14.7 m/s; the mean distance walked was 374.1±107.5 m. The mean Borg score was 12.9±2.4. Table 2 shows the main findings regarding general clinical condition and the results of the SMWT and Borg scale.

Regarding joint-related symptoms, individuals reported pain in the ankles (7.7%); knees (82.1%); hips (23.1%), and spine (48.7%). Regarding therapies for pain, 76.9% reported more than weekly usage of analgesics and/or non-steroidal anti-inflammatory drugs (NSAIDs). Table 3 summarizes these findings.

The application of the KOOS questionnaire led to the following scores in each of the five subscales: pain: 64.3±24; other symptoms: 67.2±25.5; function in daily living: 60.4±26.8; function in sport and recreation: 28.5±32.2; knee-related quality of life: 35.9±33.5. The mean Lysholm scale score was 55.3±25.4. Table 4 details these findings.

Application of SF-36 survey provided the following scores in each section: physical functioning: 41±27.4; physical role functioning: 34.6±39.2; bodily pain: 45.7±23.6; general health perceptions: 63.1±26.2; vitality: 53.5±12.1; social role functioning: 52.6±29.3; emotional role functioning: 41±44.9; mental health: 55±27.7. Table 5 summarizes the SF-36 application results.

Discussion

Gait impairment in obese individuals exerts a significant impact on routine daily activities. We observed slow gait speed and consequently low distance walked on the SMWT. Comparing our SMWT results with those observed in healthy individuals by Ziegler et al.[18] and Burr et al.,[19] we found lower distances walked. Pataky et al.[6] reported that obese women tend to have slower gait speeds and accompanying shorter stride lengths, relatively less powerful lower limbs and a poorer endurance compared to lean individuals. Comparative studies by Dufek et al.[20] and Hergenroeder et al.[21] have also shown significantly slower speeds and distances in obese individuals. Thus, gait impairment observed in our results is comparable to previous reports. SMWT has considerable advantages, since it is easy to perform, reproducible, and inexpensive. While it

TABLE 2 General clinical condition and results of the SMWT and RPE scale of the studied population.

Heart rate (bpm)	At rest: 73.1±14.3
	After SMWT: 113.5±20.3
	(p<0.0001)
Respiratory rate (brpm)	At rest: 19±4.9
	After SMWT: 24.5±8.2
	(p=0.01)
Systolic blood pressure (mmHg)	At rest: 136.7±20.9
	After SMWT: 157±22.3
	(p<0.0001)
Diastolic blood pressure (mmHg)	At rest: 95±16
	After SMWT: 91.4±15.7
	(p=0.19)
SMWT results	Speed (m/s): 63.6±14.7
	Distance (m): 374.1±107.5
RPE (Borg) score	12.9±2.4

bpm: beat per minute; brpm: breaths per minute; mmHg: millimeters of mercury; SMWT: six-minute walk test; RPE: rating of perceived exertion; m/s: meters per second; m: meters.

TABLE 3 Distribution of joint-related pain in the studied population.

Knees	32 (82.1%)
Ankles	3 (7.7%)
Hips	9 (23.1%)
Spine	19 (48.7%)
Others	4 (10.2%)

TABLE 4 Results of the KOOS questionnaire and Lysholm score.

KOOS	Pain: 64.3±24
	Other symptoms: 67.2±25.5
	Function in daily living: 60.4±26.8
	Sport and recreation: 28.5±32.2
	Knee-related quality of life: 35.9±33.5
Lysholm score	55.3±25.4

KOOS: Knee Injury and Osteoarthritis Outcome Score.

TABLE 5 Results of the SF-36 survey.

Physical functioning	41±27.4
Physical role functioning	34.6±39.2
Bodily pain	45.7±23.6
General health perceptions	63.1±26.2
Vitality	53.5±12.1
Social role functioning	52.6±29.3
Emotional role functioning	41±44.9
Mental health	55±27.7

SF-36: Short Form 36 Health Survey.

reproduces the activity of daily living, it also has a good correlation with peak oxygen uptake obtained via cardiopulmonary exercise test. However, the test does not provide insight into the mechanisms of exercise limitation.[22]

Regarding the Borg scale, this study has shown a significant perception of exertion among our obese population studied. Ziegler et al.[18] had observed lower values for this score among healthy individuals, with only 23.8% of the individuals achieving a score above 5 points; compared with the mean score of 12.9 in this study, it is reasonable to conclude that obesity has a significant impact on the perception of breathlessness. A meta-analysis that considered moderating variables such as sex, fitness level, psychological status, and mode of exercise showed that, although the validity of the scale of perceived exertion was not as high as originally reported, the relationships with physiological measures of exercise intensity remained high.[13] However, it is not free of limitations, since Joo et al.[23] reported that 80% of cardiac rehabilitation patients who were prescribed exercise at a rating of perceived exertion of 11 to 13 exercised at levels deemed to be unsafe.

Obesity is largely associated with physical impairment and joint diseases, and is currently recognized as an independent risk factor for knee osteoarthritis.[6,24-26] This study found a high prevalence of joint pain, affecting mainly the knees. In a meta-analysis, obesity led to a nearly threefold increase in the risk of knee osteoarthritis.[27] Obesity is related with these changes by means of two interconnected pathways: increased load caused by excessive weight, and systemic effects related with chronic inflammation linked to imbalance in the release of active peptides produced by the fat tissue known as adipocytokines.[22,28,29] The assessment of knee-related symptoms using KOOS score in this study has shown impact mainly regarding pain and other symptoms compared with the mean values in non-obese individuals as reported in a systematic review by Collins et al.[30] In a prospective study, Edwards et al.[31] observed significant improvement in the five domains of KOOS following weight loss, while Gudbergsen et al.[32] reported improvement in pain and function in daily living domains after weight loss. Thus, the results observed in our study regarding the KOOS methodology were similar to those previously observed in the literature. The KOOS score has undergone a substantial amount of psychometric testing, largely among populations for whom the scale was intended. Establishment of the KOOS as a reliable and valid measure across multiple languages highlights its usefulness as a patient-reported measure of knee function.[9,30] On the other hand, it also presents a few flaws, since it has not been validated for interview administration, meaning that it may not be appropriate for patients who are unable to read or write, or where telephone follow-up is necessary. Moreover, when administering the KOOS in older or less physically active individuals, the most physically demanding components of the activities of daily living (ADL) and sport/recreation subscales may not be applicable, and could result in missing data.[30]

The mean Lysholm score observed in this study was also considerably lower than that found in healthy individuals by Briggs et al. (55.3±25.4 versus 94±16.4).[33] This finding reinforces the impairment of knee function among our obese population. The Lysholm scale is a freely available measure that allows detection of changes following nonsurgical and surgical interventions. It is considered to have face validity by orthopedic surgeons. It also presents some flaws, because the items in the Lysholm scale are surgeon-derived, thus content validity from the patient's perspective cannot be assumed. The Lysholm scale was developed as a clinician-administered tool, which increases the potential for interviewer bias if the patient--reported outcome is applied as intended.[30]

We found a significantly negative overall impact of morbid obesity on quality of life assessed by the SF-36 compared to the results observed in the general population. Compared to the SF-36 scores achieved in Brazil in a study by Cruz et al.,[34] morbidly obese individuals presented significantly lower scores in all of the eight domains evaluated. Since obesity is strongly linked to several clinical comorbidities and also influences social-economical and psychological issues, this finding was expected. The results of the present study also signaled lower SF-36 scores than those observed in the general population in the United Kingdom.[35-37] Despite its wide availability and applicability, the SF-36 has also received some criticism, especially due to the possible social disability bias observed when it is obtained by means of personal interview, mainly in the mental, emotional, and vitality domains.[36] Moreover, the survey presents a low response rate in aged and

cognitively impaired individuals.[38] Nonetheless, its usage is currently widespread and it is considered a reliable tool to easily and objectively assess quality of life.[39]

This study has some limitations. First, the studied population was composed by a small number of individuals, which reduces the statistical impact of the results as well as does not make it possible to provide age and gender-related scores. Furthermore, since the study was not controlled, there was no possibility of comparison with a local matched population. The evaluation tools and questionnaires present the above cited limitations and caveats as well. Nonetheless, the findings in this study are still clearly indicative of the negative impact of obesity on physical functioning and overall quality of life. Further research, mainly in a controlled setting enrolling larger populations, is necessary to confirm these findings.

Conclusion

In our study, obesity led to significant loss of physical capacity, gait impairment, knee-related symptoms, and a negative impact on the overall quality of life.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

Statement of human and animal rights

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest

The authors declare no conflict of interest.

Resumo

Influência da obesidade mórbida sobre a capacidade física, sintomas relacionados aos joelhos e qualidade de vida geral: um estudo transversal

Objetivo: Avaliar o impacto da obesidade mórbida sobre a capacidade física, sintomas osteoarticulares e qualidade de vida global.
Método: Estudo transversal realizado em hospital universitário, envolvendo 39 indivíduos admitidos em um serviço de cirurgia bariátrica. A capacidade física foi avaliada através do teste de caminhada de 6 minutos e pela escala de percepção de esforço de Borg. Os sintomas relacionados ao joelho foram avaliados pelos escores de KOOS e Lysholm; a qualidade de vida foi avaliada por meio do questionário SF-36.
Resultados: No teste de caminhada de 6 minutos, a distância média foi de 374,1±107,5 m. O escore médio de Borg foi 12,9±2,4. Os seguintes escores foram observados no KOOS: dor (64,3±24); outros sintomas (67,2±25,5); atividades da vida diária (60,4±26,8); atividades esportivas e lazer (28,5±32,2); qualidade de vida (35,9±33,5); o escore de Lysholm médio foi 55,3±25,4. O SF-36 mostrou estes escores: capacidade funcional (41±27,4); limitação por aspectos físicos (34,6±39,2); dor (45,7±23,6); estado geral de saúde (63,1±26,2); vitalidade (53,5±12,1); aspectos sociais (52,6±29,3); aspectos emocionais (41±44,9); saúde mental (55±27,7).
Conclusão: A obesidade levou à perda significativa de capacidade física, a prejuízo à marcha, a sintomas relacionados ao joelho e a impacto negativo sobre a qualidade de vida global.

Palavras-chave: obesidade, qualidade de vida, joelho, artropatias, artralgia.

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Regulation of muscle plasticity and trophism by fatty acids: A short review

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Study conducted at Universidade de São Paulo, Universidade Estadual do Ceará and Universidade Cruzeiro do Sul, Brazil

Article received: 6/17/2016
Accepted for publication: 6/26/2016

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http://dx.doi.org/10.1590/1806-9282.63.02.148

Summary

The skeletal muscle tissue has a remarkable ability to alter its plastic structural and functional properties after a harmful stimulus, regulating the expression of proteins in complex events such as muscle regeneration. In this context, considering that potential therapeutic agents have been widely studied, nutritional strategies have been investigated in order to improve the regenerative capacity of skeletal muscle. There is evidence of the modulatory action of fatty acids, such that oleic and linoleic acids, that are abundant in Western diets, on muscle function and trophism. Thus, fatty acids appear to be potential candidates to promote or impair the recovery of muscle mass and function during regeneration, since they modulate intracellular pathways that regulate myogenesis. This study is the first to describe and discuss the effect of fatty acids on muscle plasticity and trophism, with emphasis on skeletal muscle regeneration and in vitro differentiation of muscle cells.

Keywords: cell differentiation, muscle repair, skeletal muscle, satellite cells, fatty acids.

Introduction

Skeletal muscles have the plastic ability to adapt to the intrinsic and extrinsic demands of the environment. Such adaptive potential is attributed to the population of stem cells resident in adult skeletal muscle, known as satellite cells.[1] These are mononuclear and undifferentiated satellite cells located between the basal lamina and the sarcolemma of a muscle fiber, which proliferate, differentiate and fuse leading to the formation of a new myofiber and, thus, the reconstitution of the contractile apparatus.[2,3]

The process of muscle regeneration is triggered by a noxious stimulus whose nature can be mechanical,[3] chemical,[4] or thermal.[5] Skeletal muscle repair, triggered by sarcolemmal rupture and increased vascular permeability, involves cellular and molecular events that begin with increased calcium influx into the intracellular environment causing proteolysis dependent on that cation, necrosis of damaged tissue, and activation of inflammatory response at the lesion site. This phase is followed by the production of extracellular matrix proteins, revascularization and concomitant activation of myogenic cells.[2,3,5,6]

Several groups have investigated therapeutic strategies to accelerate the skeletal muscle repair process after injury. There is evidence that fatty acids modulate muscle function and trophism.[7-11] Our article is the first to describe and discuss the effects of fatty acids, especially oleic and linoleic acids, which are the most abundant fatty acids in Western diets, on muscle plasticity and trophism, with emphasis on skeletal muscle regeneration and in vitro differentiation of muscle cells. This is the main focus of our review. The searches were carried out in five bibliographic databases: PubMed, Web of Science, Scientific Electronic Library Online (SciELO), Excerpta Medica database (Embase), and Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS). References found in duplicity were excluded. Articles written in English and Portuguese were selected. As for the terms, we considered that there are differences in the indexing processes among the bibliographic databases and, therefore, we opted for searching free terms, and did not use controlled vocabulary (descriptors). In this way, more references were retrieved, guaranteeing that most published works were detected. The terms fatty acids; oleic and linoleic acid; muscle function and trophism; plasticity and muscle trophism; skeletal muscle regeneration; skeletal muscle repair; muscle satellite cells; and muscle cell differentiation were combined, as proposed by Sin et al.[12]

Skeletal muscle satellite cells

Initially described in frog muscle fibers,[13] skeletal muscle satellite cells, undifferentiated and mononucleated, have this name because of their anatomical positioning at the periphery of the muscle fiber, between the basal lamina and the plasma membrane. They represent between 2 and 10% of the total myonuclei per muscle fiber and total 2 x 10^5 to 1 x 10^6 cells per gram of muscle.[14]

Muscle fibers are differentiated cells, unable to undergo division. The ability of muscles to self-repair has been attributed to satellite cells, which have high mitotic capacity, contributing to the maintenance and regeneration of adult skeletal muscle.[14]

In his article, Mauro[13] defined these satellite cells as "myoblastic cells in the adult organism, which failed to merge with other myoblasts. These cells are juxtaposed to recapitulate the embryonic development of skeletal muscle fibers". Subsequently, studies have shown that asymmetric divisions of satellite cells generate myogenic cells, which originate myoblasts, myocytes and myofibers.[15] Symmetric divisions, on the other hand, generate new satellite cells that expand the number of these cells, a process known as self-renewal. Multiple factors are part of this complex network of satellite cell growth and differentiation, governing the cell cycle progression and/or the return to a quiescent state (G0).[15]

In the absence of stimuli in the muscle tissue, the satellite cells remain in the G0 state. Once activated, these cells initiate the cell cycle, proliferate and express myogenic growth and differentiation markers.[1] Thus, a balance between G0 and the active state (self-renewal or myogenic differentiation) is indispensable for the conservation of muscle tissue.[16] Studies have shown that the number of satellite cells remains constant even after multiple activations.[17] Currently, many intrinsic and extrinsic factors that control the satellite cell function have been discovered. In all, these studies have demonstrated that there are specific cell cycle activations and inhibitions, and progression to myogeny.

Transcription factor PAX7 (meaning paired box) was the first marker identified in satellite cells in quiescent state, being activated during proliferation. It has a key function in the maintenance of the G0 state and in the prevention of early myogenic differentiation.[15] Studies have demonstrated the complete loss of muscle regenerative capacity in PAX7 knock-out mice ($PAX7^{-/-}$).[18,19]

In this context, increasing evidence shows that satellite cells are composed of two different populations that regulate the cell cycle: (1) those with stem cell potential, undifferentiated and which remain in G0 state during myogenic progression, and (2) those with potential for myogenic differentiation.[20]

After muscle injury, satellite cells are activated, initiating the expression of regulatory factors of myogenesis, such as myoblast differentiation (MYOD) and/or myogenic factor 5 (MYF5).[21] MYOD is expressed in extremely low amounts and is essentially undetectable in quiescent satellite cells. This protein marks the compromise of myoblasts with the myogenic lineage. In this context, the concomitant expression of MYOD and MYF5 is vital for the formation of myotubes and myofibers.[1,22]

Promotion of myofibroblast restoration and reorganization results from a decrease in PAX7 expression, cell cycle arrest and increased expression of myogenin (MYOG)[23] and myogenic regulatory factor 4 (MRF4),[24] both members of the superfamily of basic helix-loop-helix (bHLH) transcription factors. These factors are specific to skeletal muscle, being expressed at distinct moments during myogenesis. They have key functions in myogenic specification, muscle differentiation and maintenance during muscle development and regeneration[22] (Figure 1).

Effects of fatty acids on skeletal muscle trophism and regeneration

The regulation of trophism and muscle regeneration involves the coordinated action of various cell types in response to local and systemic signals. It is slow and often incomplete depending on its severity, leading to loss of function.[26] Thus, the discovery of new dietary strategies to improve skeletal muscle regeneration capacity can be a powerful tool for the development of new nutritional therapies in order to accelerate regenerative processes and/or reduce the consequences of incomplete repair and extensive fibrosis deposited in the skeletal muscle, as occurs after severe muscle injuries.

The composition of phospholipids in the plasma membrane has a crucial influence on cell growth and metabolic activity. In the last two decades, it has been suggested that the lipid composition of the diet influences the fatty acid profile of the serum and the lipid content of the plasma membrane.[27-29] In fact, it has been shown that the length of the fatty acid chain and the degree of saturation or unsaturation alter the fluidity and activity of several membrane-bound proteins.[30,31]

In this context, few studies have evaluated the involvement of fatty acids in muscle trophism and myogenesis. Muscle differentiation is known to be accompanied by important metabolic changes, such as increased expression of genes related to the metabolism of carbohydrates and amino acids.[32,33] In this context, some nutritional strategies were evaluated in models of muscle injury in rodents. Pereira et al.[32] found that supplementation with

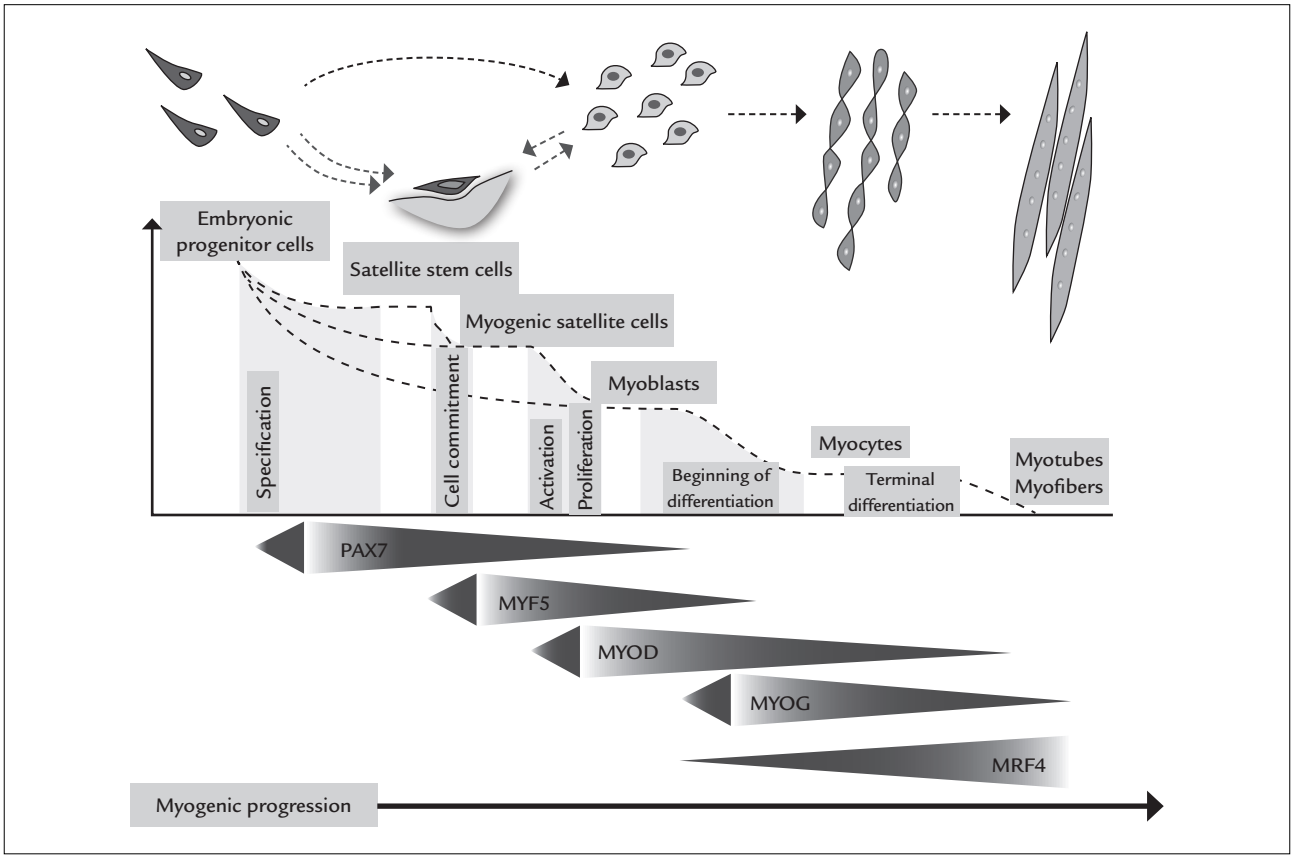

FIGURE 1 Temporal activation of regulatory factors of myogenic differentiation. In skeletal muscle, satellite cells in the quiescent state or activated during proliferation express the paired-box 7 (PAX7) transcription factor. These cells have the ability to proliferate, self-renew, differentiate and fuse with newly formed myotubes or existing myofibers after stimuli such as muscle injury. Differentiation of satellite cells involves increased expression of basic helix-loop-helix (bHLH), myogenic factor 5 (MYF5) and myoblast determination (MYOD) transcription factors. Myogenin (MYOG), a transcription factor essential for myogenesis and skeletal muscle repair, is highly expressed during the formation of myocytes by the fusion of myoblasts. Myogenic regulatory factor 4 (MRF4) transcription factor is, then, activated during terminal differentiation and formation of myocytes and myotubes. (Modified from Wang and Rudnicki).[25]

leucine during the muscle regeneration process accelerates the repair of connective tissue in the anterior tibial muscle of rats. Baptista et al.[33] investigated the effect of supplementation with leucine and HMB (β-hydroxy-β-methylbutyrate) on the ubiquitin-muscle proteasome system under different sarcopenia conditions in rats, demonstrating the anti-atrophic effect of leucine.[32,33]

Oleic acid (monounsaturated 18:1 (n-9)) and linoleic acid (polyunsaturated 18:2 (n-6)) are the most abundant fatty acids in Western diets. Oleic acid is found mainly in olive oil, while linoleic acid is found in soy, sunflower and corn oils.[34] Using both isolated muscle cells and animal models, Salvadó et al.[35] demonstrated that oleic acid is capable of reversing the structural and metabolic changes in skeletal muscle induced by palmitic acid. In another study, the increase in docosahexaenoic acid (DHA) content (22:6 (n-3)) in the gastrocnemius muscle through supplementation with fish oil for 21 days is suggestive that this fatty acid attenuates lipopolysaccharide-induced muscle atrophy (LPS).[36] Other studies suggest that the reduction in DHA content impairs calcium homeostasis in the skeletal muscle cell.[37] Tuazon and Henderson[38] observed that increases in linoleic acid content and decline in DHA content in muscle phospholipids were positively correlated with increased creatine kinase activity, combined with decline in muscle grip strength of dystrophin knock-out animals. Another study shows that there is an inverse relationship between the concentration of oleic and linoleic acid in skeletal muscle.[39]

It has been demonstrated that some fatty acids, such as oleic and linoleic acids, exert pro-proliferative effects on vascular smooth muscle, and may regulate muscle

growth.[40] Perdiconi et al.[41] observed an increase in total phospholipid content during muscle differentiation. Myoblasts predominantly synthesize triacylglycerols, while myotubes synthesize phospholipids.[42] The fusion of myoblasts can also be regulated by factors that alter the fluidity of the plasma membrane, such as temperature and lipid composition.[43,44]

The fatty acid composition of phospholipids determines the physicochemical properties of the plasma membrane and, to a large extent, its asymmetry, fluidity, plasticity, organization and occurrence of microdomains.[45] The incorporation of omega-3 or -6 polyunsaturated fatty acids into membrane phospholipids affects lipid and protein interactions in the membrane, in addition to the physical properties mentioned above.[45] For example, the decrease in insulin sensitivity in skeletal muscle has been associated with a decrease in the proportion of polyunsaturated fatty acids in membrane phospholipids.[46]

There is evidence that intermediate products of fatty acid metabolism are important for the survival, proliferation, differentiation, and fusion of myoblasts.[47] Rodeman and Goldberg[48] suggested that lipid metabolites derived from polyunsaturated fatty acids, such as arachidonic acid, accelerate protein synthesis, fusion and growth of muscle cells in different animal models.

Oxidation of fatty acids is significantly higher in myotubes compared to myoblasts, and mitochondrial biogenesis is necessary for skeletal muscle differentiation.[42] The content of triacylglycerols decreases by more than 50% during myogenesis,[41] and the inhibition of mitochondrial respiration compromises myogenic differentiation and the formation of myotubes.[49] Leptin knock-out mice (ob/ob), which have high concentrations of plasma fatty acids, present deficient muscle regeneration.[50] In addition, a hyperlipidic diet compromises muscle regeneration in mice,[51] possibly by the effect of saturated fatty acids.

Pinheiro[43] observed that there is an increase in the synthesis of oleic and arachidonic acids during myogenesis in vitro. The addition of arachidonic and linoleic acids to the culture medium increases the proliferation of satellite cells, as assessed by the incorporation of ^{14}Carbon-labeled thymidine. The author also observed that supplementation with linoleic acid for 20 days in dystrophic mice (mdx) significantly improves the strength of the gastrocnemius muscle of these animals, suggesting a possible trophic effect of this fatty acid.[43] On the other hand, the diet rich in saturated fatty acid and linoleic acid causes insulin resistance and imbalance of oxidative components in skeletal muscle, resulting in oxidative stress. Pariza et al.[52] demonstrated in vivo that supplementation with conjugated linoleic acid (0.3% of diet) causes a decrease in fat mass and an increase in fat-free lean mass in rodents.

Our group evaluated the effect of treatment with oleic and linoleic acids (0.44 g per kg body weight) for four weeks on lacerated gastrocnemius muscle regeneration in rats (unpublished data). Laceration *per se* causes an increase in the oleic/stearic and palmitoleic/palmitic ratio indicators of the desaturase activity and promotes a reduction of specific isotonic and specific absolute tetanic forces. There is also a drop in resistance to fatigue and an increase in the area of fibrous tissue. These findings indicate incomplete regeneration and partial recovery of the contractile function of the injured muscle. Linoleic acid supplementation decreases the mass, specific isotonic strength, fatigue resistance, and cross-sectional area of the contralateral and injured gastrocnemius muscle fibers, as well as increases the area of fibrous tissue in the injured muscle. Supplementation with oleic acid, on the other hand, does not modify the mass and the cross-sectional area of the fibers of the gastrocnemius muscle; it suppresses the decrease in specific isotonic force and the increase in the area of fibrous tissue induced by the injury, prevents tetanic forces (absolute and specific), and increases the resistance to fatigue in the contralateral and injured gastrocnemius muscles. Based on these findings, we conclude that supplementation with linoleic acid compromises the regeneration of the injured skeletal muscle, causing muscle mass reduction, fibrous tissue elevation, and, consequently, impairment of contractile function. Oleic acid, in turn, attenuates incomplete repair actions, optimizing the regenerative capacity and the contractile function of the injured muscle.

Effects of fatty acids on muscle cell differentiation

The effects of fatty acids on fibroblast proliferation[53,54] and myogenic differentiation in isolated cells have been investigated.[7-11] In 1978, Horwitz et al.[55] observed that the fatty acids added to the culture medium have a stimulatory effect on the fusion of embryonic myoblasts. The authors observed that the lipid composition of the membrane influences the proliferation and fusion of myoblasts and, consequently, the formation of multinucleated myotubes. In 1985, Allen et al.[56] observed that linoleic acid and insulin stimulate the differentiation of satellite cells by regulating cell fusion. Incubation of the cells with linoleic acid (1 µg/mL) raises the degree of differentiation and fusion of satellite cells, without, however, changing the total number of cells. The authors also

observed that the presence of mitogenic agents in culture medium and the subsequent increase in proliferation prevent differentiation.

Lu et al.[57] observed that adding fatty acids to the culture medium induced proliferation of vascular smooth muscle, and that oleic acid had a more pronounced effect on the stimulation of proliferation, an effect associated with the activation of protein kinase C (PKC). Hurley et al.[8] compared the effect of different fatty acids on the differentiation of L6 myoblast in vitro. To assess the degree of differentiation, the authors quantified protein and DNA contents, as well as creatine kinase activity (CK/DNA). The effects on differentiation were accompanied by analysis of peroxisome proliferator-activated receptor alpha and gamma (PPAR-α and -γ) receptor activity to establish the possible association of these transcription factors with the differentiation process. They observed that linoleic acid stimulates differentiation at low concentrations (50 μM) and oleic acid at all concentrations tested (12.5 to 100 μM) without the involvement of the activation of the PPARs evaluated.

Lee et al.[7] investigated the effect of fatty acids on the proliferation and differentiation of C2C12 myoblasts, as well as the possible involvement of the mitogen activated protein kinases (MAPK) in this process. The authors have found that linoleic and oleic acids increase cell proliferation and differentiation, with phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) and c-Jun N-terminal kinases (JNK) occurring during proliferation, but not during differentiation. Markworth and Cameron-Smith[9] evaluated the effect of arachidonic acid treatment on C2C12 myoblasts and observed that arachidonic acid stimulates the growth of myoblasts in a dose-dependent manner at concentrations between 1.6 and 25 μM. There was an increase in myonuclei during myogenesis, regardless of changes in cell density or extent of myogenic differentiation. To verify the effect of arachidonic acid treatment on hypertrophy in myotubes, the authors cultured C2C12 myoblasts in differentiation medium for 72 hours. Then, they added arachidonic acid (25 μM) to the medium containing the already differentiated myotubes. Researchers have found that hypertrophy is greater in arachidonic acid-treated myotubes compared to untreated cells.

Briolay et al.[10] showed that oleic (18:1 (n-9)), arachidonic (20:4 (n-6)), eicosapentaenoic (EPA) (20:5 (n-3)) and DHA (22:6 (n-3)) (20 μM) acids stimulate the myogenic differentiation of L6 myoblast. These fatty acids alter the lipid composition of the membrane and, during myogenic differentiation, promote phosphorylation of ribosomal protein S6 kinase beta-1 – 70 KDa (p70S6K1) and activation of mammalian target of rapamycin complex 1 (mTORC1), an important cell cycle regulator and protein synthesis, without alteration of Akt phosphorylation. These results support the proposition that the fatty acid composition of the plasma membrane can control the activity of complex signaling pathways of myogenic differentiation. In this context, the treatment of isolated myoblasts with arachidonic acid rapidly stimulates protein turnover (synthesis and degradation)[48] (Figure 2).

Our group also assessed the effect of oleic and linoleic fatty acids (100 μM) on myoblast differentiation, myotubes growth and fibroblast proliferation in primary culture (data still unpublished). Treatment of fibroblasts with linoleic acid decreases mRNA expression of proliferating cell nuclear antigen (PCNA), collagen and fibronectin. Oleic acid, in turn, increases the content of MYOD mRNA in myoblasts, increases desmin in previously differentiated myotubes, and inhibits mRNA expression of PCNA, collagen and fibronectin in fibroblasts. We conclude that oleic acid, in vitro, has a modulatory effect on the differentiation of satellite stem cells and on the growth and maturation of myotubes.

Final considerations

Recently, studies have revealed significant advances in the knowledge of mechanisms involved in the activation, proliferation and differentiation of muscle cells, as well as on fundamental processes of muscle trophism and plasticity. Dietary strategies have been investigated with a view to improving skeletal muscle regenerative capacity after injury. In this context, different fatty acids, such as oleic and linoleic acids, which are abundant in Western diets, have demonstrated in vitro modulatory effects on muscle cell differentiation, and in vivo effects on muscle plasticity and trophism, with an emphasis on regeneration of skeletal muscle. Recent evidence on the regulatory action of fatty acids on muscle function and muscle mass has been described and discussed in this review.

Acknowledgments

This study had financial support from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Instituto Nacional de Ciência e Tecnologia em Obesidade e Diabetes (INOD), Pró-reitoria de Pesquisa da Universidade de São Paulo (PRP/USP) and Pró-reitoria de Pós-graduação e Pesquisa da Universidade Cruzeiro do Sul (PRPGP/Universidade Cruzeiro do Sul).

Conflict of interest

The authors declare no conflict of interest.

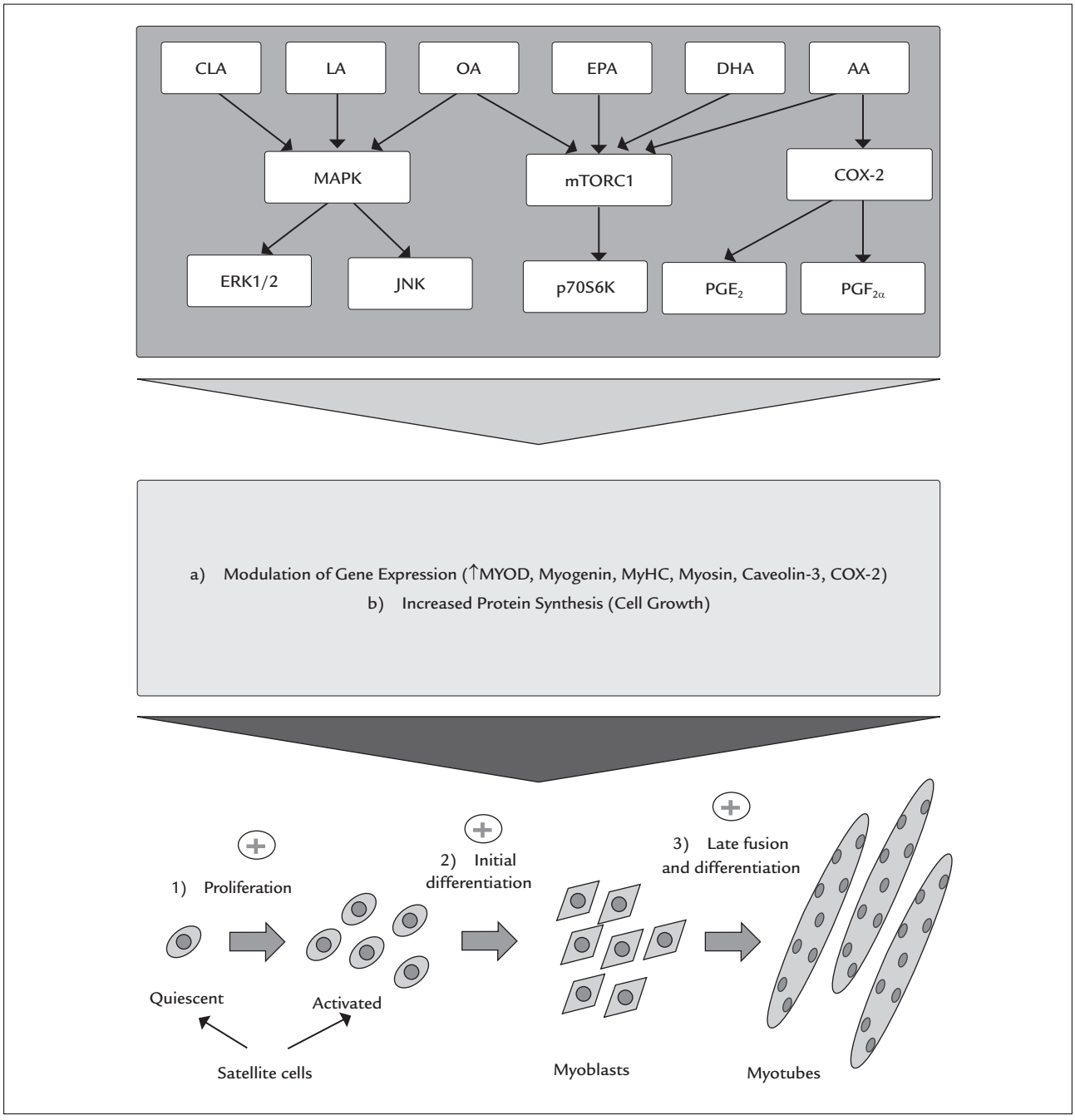

FIGURE 2 Mechanisms possibly involved in the action of different fatty acids on the proliferation and differentiation of skeletal muscle cells. For details, see "Effects of fatty acids on muscle cell differentiation".

CLA: conjugated linoleic acid; LA: linoleic acid; OA: oleic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; AA: arachidonic acid; MAPK: mitogen activated protein kinases; ERK1/2: extracellular signal-regulated kinases 1 and 2; JNK: c-Jun N-terminal kinases; mTORC1: mammalian target of rapamycin complex 1; p70S6K: ribosomal protein S6 kinase – 70 KDa; COX-2: cyclo-oxygenase-2; PGE_2: prostaglandin E_2; $PGF_{2\alpha}$: prostaglandin $F_{2\alpha}$; MYOD: myogenic differentiation; MyHC: myosin heavy chain.

Resumo

Regulação da plasticidade e do trofismo muscular pelos ácidos graxos: uma breve revisão

O tecido muscular esquelético possui a notável capacidade plástica de alterar suas propriedades estruturais e funcionais após um estímulo lesivo, regulando a expressão de proteínas durante eventos complexos como a regeneração muscular. Nesse contexto, considerando que possíveis agentes terapêuticos vêm sendo amplamente estudados, estratégias nutricionais têm sido investigadas na perspectiva de melhorar a capacidade regenerativa do músculo esquelético. Há evidências da ação modulatória dos ácidos graxos, como os ácidos oleico e linoleico, que são abundantes nas dietas ocidentais, sobre a função muscular e o trofismo. Nesse sentido, os ácidos graxos parecem ser potenciais candidatos para promover ou prejudicar a recuperação da massa e a função muscular durante a regeneração, uma vez que modulam vias intracelulares reguladoras da miogênese. Este trabalho é o primeiro a descrever e discutir o efeito dos ácidos graxos sobre a plasticidade e o trofismo muscular, com ênfase na regeneração do músculo esquelético e na diferenciação de células musculares *in vitro*.

Palavras-chave: diferenciação celular, reparo muscular, músculo esquelético, células satélites, ácidos graxos.

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Hypomagnesemia and its relation with chronic low-grade inflammation in obesity

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Article received: 5/23/2016
Accepted for publication: 5/31/2016

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http://dx.doi.org/10.1590/1806-9282.63.02.156

Summary

Introduction: The accumulation of visceral fat in obesity is associated with excessive production of proinflammatory adipokines, which contributes to low-grade chronic inflammation state. Moreover, the literature has shown that mineral deficiency, in particular of magnesium, has important role in the pathogenesis of this metabolic disorder with relevant clinical repercussions.
Objective: To bring updated information about the participation of hypomagnesemia in the manifestation of low-grade chronic inflammation in obese individuals.
Method: Articles published in PubMed, SciELO, LILACS and ScienceDirect, using the following keywords: "obesity," "magnesium" and "low grade inflammation."
Results: Scientific evidence suggests that magnesium deficiency favors the manifestation of low-grade chronic inflammation in obese subjects.
Conclusion: From literature data, it is evident the participation of magnesium through biochemical and metabolic reactions in protecting against this metabolic disorder present in obesity.

Keywords: obesity, magnesium, low-grade inflammation.

Introduction

White adipose tissue is the main energy source in the body, mobilizing fatty acids according to metabolic need.[1] In excessive amounts, this tissue produces proinflammatory adipokines, a process influenced by the anatomical location of fat deposits. Visceral fat, being metabolically more active, favors an increase in the production of these substances, contributing to chronic low-grade inflammation in obesity.[2,3]

Low-grade chronic inflammation differs from other types of inflammation as it leads to latent tissue damage for extended periods of time, lasting for decades, silently.[1,2] Studies have shown that in obese individuals the inflammatory state favors an increase in the formation of reactive oxygen species that can lead to an overload of the antioxidant defense system, contributing to the manifestation of oxidative stress and, consequently, cell damage and death.[4,5]

Biochemical and nutritional disorders present in obese individuals are being extensively investigated in order to elucidate the mechanisms involved in the pathogenesis of obesity. In this sense, minerals have been the subject of extensive research in order to identify their relation with metabolic disorders.

Magnesium in particular has attracted great interest from researchers as it plays a role in glucose metabolism, insulin homeostasis, synthesis of adenosine triphosphate, proteins and nucleic acids, as well as in membrane stability and regulation of hormonal and immunological function.[6,7]

Magnesium deficiency is characterized as a nutritional problem that leads to changes in the cellular function and biological activity of the molecules, and may contribute to the onset of metabolic disorders related to the inflammatory process, especially in obese individuals, who present low serum and dietary concentrations of this mineral.[8-10]

In view of the biochemical and metabolic aspects of magnesium, as well as the importance of the functions of this mineral, particularly in mechanisms involved in

the pathogenesis of chronic diseases such as obesity, the objective of this review was to bring updated information on the participation of hypomagnesemia in the manifestation of low-grade chronic inflammation in obese individuals.

Method

The literature search was carried out in PubMed, SciELO, LILACS and ScienceDirect databases with no restrictions as to year of publication, considering the following inclusion criterion: studies on the metabolic and physiological aspects of magnesium, which presented relevant aspects on the role of this mineral in the manifestation of chronic low-grade inflammation in obese individuals. The articles were selected based on originality and relevance, taking into account the accuracy and adequacy of the experimental design and the sample number. Established and recent works were preferably used.

The search for bibliographic references was performed using the following keywords: "obesity," "magnesium" and "low grade inflammation." The literature search included the following types of studies: randomized or quasi-randomized controlled clinical trials, case-control study, and review articles.

Metabolic and physiological aspects of magnesium

Magnesium is the second most abundant intracellular cation and is involved in about 300 biochemical reactions related to anabolic and catabolic actions in the body, such as glycolysis and protein and lipid metabolism.[11] This mineral contributes to increase the production of intracellular adenosine triphosphate and the use of glucose, acting as a cofactor in all reactions that involve energy transfer.[12]

On average, the body of an adult contains 1 mole of magnesium. About half of the mineral content is present in the bone and the other half in soft tissues. More precisely, 0.3% of the total is found in serum, 0.5% in erythrocytes, 19.3% in soft tissues, 27% in muscles, and 52.9% in bones. In serum about one-third of the magnesium is bound to proteins. Of this total, 25% is bound to albumin and 8% to globulins. Of the remaining magnesium, about 80% is in the form of free ion (55% of total magnesium) and about 20% is combined with phosphate, citrate and other compounds.[13]

Magnesium homeostasis in the body is dependent on the amount ingested, intestinal absorption, renal excretion and need presented by various tissues.[11] About 25 to 60% of ingested magnesium is absorbed into the gastrointestinal tract by passive or active transport. The transport of this nutrient through the paracellular pathway is responsible for 80 to 90% of its absorption, which occurs predominantly between microvilli of the small intestine through simple diffusion, and this process is stimulated when intraluminal concentrations of this mineral are high. This absorption pathway occurs mainly in the ileum and distal parts of the jejunum, where the permeability to this ion is greater. This is because in these sites there is a low expression of claudin proteins 1, 3, 4, 5 and 8, which participate in the formation of paracellular barriers and pores, regulating the passage of substances through the epithelium.[14-16]

However, in the case of low intraluminal concentrations, the magnesium is absorbed through the action of specific transporters belonging to the family called transient receptor potential channel of melastatin type (TRPM6 and 7), and this process occurs by the active absorption of sodium ions, followed by water.[17] This transport requires strict regulation since magnesium ions cross two cell membranes. The active absorption of the mineral occurs mainly in the colon and, to a lesser extent, in the jejunum and ileum.[14-16]

It is important to emphasize that excessive calorie intake promotes an increase in the intestinal absorption of magnesium, since the mechanism involved in this process is energy dependent. However, the absorption of this mineral can be impaired in the presence of lipids, phosphorus, phytates and oxalate. Diets low in protein (< 30 g/day) also slow the absorption of magnesium.[18,19]

The kidneys are the main excreting organs involved in magnesium homeostasis, and 70% of the entire content of filtered mineral is reabsorbed in the thick ascending branch of the loop of Henle via the paracellular route. The driving force for magnesium reabsorption is positive transluminal epithelial tension generated by the recycling of potassium through the apical membrane, which is linked with sodium, water and calcium. In the distal convoluted tubule, magnesium transport mainly occurs by active process mediated by TRPM6, and is characterized by negative and highly resistant luminal tension, a specific process that does not depend on calcium absorption.[20,21]

In a situation of reduced oral intake of magnesium, the kidneys are able to reduce their excretion. The other routes of magnesium excretion are feces and sweat, with the fecal concentration of the mineral being about 150 to 200 mg/day, while sweating contributes about 15 mg daily loss.[18,22] The balance of magnesium in the body is maintained by the regulation of urinary excretion, which can be exacerbated by the action of thyroid hormones, acidosis, aldosterone, and depletion of phosphate and potassium. On the other hand, calcitonin, glucagon and parathyroid hormone increase reabsorption of glomerular filtrate.[23]

The evaluation of nutritional status relative to magnesium can be obtained by assessing its contents in plasma, erythrocyte, urine and diet. Plasma magnesium has been widely used. However, this marker does not reflect its total content since, even after reduction in mineral intake, plasma concentrations remain constant for a long period of time.[6,11] The reference values for normal plasma magnesium concentrations are between 0.75 and 1.05 mmol/L.[18,19]

Erythrocyte magnesium concentration is approximately 2.5 mmol/L and since it has a half-life of 120 days, medium and long-term evaluations of the mineral's stock in the body can be performed.[24,25] As for urinary magnesium, approximately 3 to 4 mmol of the nutrient is lost daily through this excretion route. Urine is considered a good indicator for recent changes in nutritional status regarding magnesium, because in cases of stock depletion, excretion is reduced by renal reabsorption mechanisms to maintain its homeostasis in the body.[24]

The main food sources of magnesium are whole grains, dark green vegetables, legumes, walnuts, seeds, chestnuts and almonds.[26] The dietary recommendation of this mineral is 400 to 420 and 310 to 320 mg daily for adult men and women, respectively.[27]

Hypomagnesemia and low-grade chronic inflammation

The literature has shown that the diet of obese individuals has reduced magnesium content, which is a nutritional problem of great relevance.[28,29] Huang et al.[30] and Song et al.[31] found that dietary intake of magnesium is inversely proportional to body mass index, waist circumference, and body fat percentage.

The reduced intake of magnesium by obese individuals can be explained mainly by the high consumption of processed foods containing low magnesium and by the reduced intake of food sources of magnesium, which seems to contribute to the reduction of its concentrations in the blood compartments.[15]

Studies have found reduced plasma concentrations of magnesium in obese individuals.[32,33] Guerrero-Romero and Rodríguez-Morán[34] have shown that individuals with normal body weight but metabolically obese exhibit reduced serum magnesium concentrations compared to the obese who are metabolically healthy. Table 1 shows data on the status of magnesium in obese individuals, as well as its participation in chronic low-grade inflammation.

Magnesium deficiency seems to affect the activation of proinflammatory pathways in obese individuals.[40] In this regard, several researchers have observed that the reduced intake of this mineral and its low serum concentration are strongly related to the increase in the plasma concentration of inflammatory biomarkers, such as C-reactive protein, tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6).[26,33,41]

Nielsen et al.[42] found that magnesium intake in amounts below estimated average requirement (EAR) shows a positive correlation with plasma C-reactive protein and body mass index in adults. Guerrero-Romero et al.[43] found severe hypomagnesemia in individuals with metabolic syndrome, being this parameter strongly related to serum concentrations of C-reactive protein and TNF-α.

A study conducted by Oliveira et al.[35] revealed reduced dietary magnesium content and urinary excretion in obese women. In addition, a positive correlation was observed between urinary magnesium and serum concentrations of C-reactive protein in these patients, suggesting the influence of hypomagnesuria on this inflammatory marker.

Reduced concentrations of magnesium in plasma compromise its intracellular homeostasis and contribute to the development of a proinflammatory state through overproduction and release of cytokines such as interleukin 1β (IL-1β) and TNF-α, and increased serum concentrations of neuropeptides.[11,44,45]

It is important to mention that the mechanisms involved in the inflammatory response present in magnesium deficient obese individuals are not yet clearly elucidated. However, according to the literature, the opening of calcium channels and the activation of N-methyl-D-aspartate (NMDA) receptors, as well as the priming of phagocytic cells, induce the entry of calcium into the cell, release of neurotransmitters, such as substance P, membrane oxidation and activation of nuclear transcription factor kappa B (NF-kB), which favors the inflammatory process.[22,45,46]

The inflammatory response is mainly related to the change in the extracellular concentration of magnesium, since the deficiency of this mineral reduces its plasma concentrations but does not alter its intracellular concentration. Thus, it is important to emphasize the action of magnesium as a natural calcium antagonist and that the reduction of magnesium in the extracellular compartment induces an increase in the concentration of intracellular calcium, favoring the activation of phagocytic cells and the production of cytokines.[35,46]

One of the mechanisms that seem to justify the increase of intracellular calcium is that of NMDA receptor activation. The decline in extracellular magnesium decreases the concentrations of amino acids such as glutamate needed to activate this receptor. Activation of NMDA, in turn, allows the influx of calcium into the neural cells. In the presence of obesity, this effect can be accentuated

TABLE 1 Studies evaluating the status of magnesium in obese individuals or their relationship to chronic low-grade inflammation.

Author(s)	Study design	Results
Oliveira et al.[35]	65 obese and 66 non-obese women Plasma, erythrocyte and urinary magnesium and C-reactive protein	Obese women had plasma and erythrocyte magnesium concentrations similar to the control group Obese women had lower than normal values of magnesium in the urine Correlation between urinary concentrations of magnesium and C-reactive protein
Farhangi et al.[9]	40 obese and 42 non-obese women Serum magnesium	Obese women had lower serum magnesium concentrations than the control group
Cruz et al.[10]	55 obese and 59 non-obese women Plasma, erythrocyte and urinary magnesium	Obese women had plasma and erythrocyte magnesium concentrations similar to the control group Obese women presented values of urinary magnesium lower than normal
Zemva e Zemva[36]	32 obese and 32 non-obese individuals Plasma and erythrocyte magnesium	Obese individuals had lower plasma and erythrocyte magnesium values compared to the control group
Corica et al.[37]	19 obese normotensive, 19 obese hypertensive, and 15 non-obese individuals Plasma, erythrocyte and platelet magnesium	Lower plasma, erythrocyte and platelet magnesium levels in the normotensive and hypertensive obese group compared to the control group
Bertinato et al.[38]	276 southern Asian and 315 Caucasian individuals. Serum magnesium	Obese women had lower serum magnesium values than normal and overweight women
Suliburska et al.[39]	78 obese and 20 non-obese adolescents Serum magnesium	Obese adolescents presented lower serum magnesium compared to the control group
Song et al.[41]	11,686 women aged ≥ 45 years Magnesium content in diet and plasma C-reactive protein	Inverse association between plasma C-reactive protein concentrations and dietary magnesium content after adjustment for age and body mass index
Guerrero-Romero et al.[43]	51 women and 47 men Serum magnesium, TNF-α and C-reactive protein	Severe hypomagnesemia in individuals with metabolic syndrome, a parameter strongly related to the serum concentrations of C-reactive protein and TNF-α
Moslehi et al.[57]	69 overweight women Serum magnesium and C-reactive protein, and plasma IL-6 Supplementation with 250 mg/day of magnesium oxide for 8 weeks	Magnesium serum concentrations were inversely correlated with C-reactive protein before supplementation However, supplementation with magnesium did not alter the serum concentrations of this mineral, and did not reduce the levels of C-reactive protein and IL-6
Rodriguez-Hernandez et al.[58]	38 obese women Serum magnesium and C-reactive protein Supplementation with 450 mg/day of magnesium chloride for 4 weeks	No reduction in C-reactive protein concentrations was observed in obese women treated with supplements
Simental-Mendía et al.[59]	62 men and women diagnosed as pre-diabetic Serum magnesium and C-reactive protein Supplementation with 382 mg/day of magnesium chloride for 12 weeks	Oral magnesium supplementation reduced levels of C-reactive protein in subjects with pre-diabetes and hypomagnesemia
Niranjan et al.[60]	62 obese children and 60 controls Serum magnesium and C-reactive protein	Reduced serum magnesium concentrations were seen in the case group compared to the control, as well as of C-reactive protein

by leptin, a hormone that also favors the receptor's activation.[40] Thus, excessive calcium influx into the neuronal tissue promotes the release of neurotransmitters, such as substance P, which triggers an inflammatory response through the release of cytokines, histamine and free radicals[47] (Figure 1).

In adipose tissue, the increase of the intracellular calcium content derives from the opening of the L-type calcium channels, which is regulated by magnesium binding sites. In the presence of deficiency of this nutrient, the blockage of these channels is compromised, increasing the influx of calcium to the adipose cells. Excess intracellular calcium, in turn, results in the activation of calcium-dependent processes, such as the release of proinflammatory cytokines. Note that one of the major events in the calcium-mediated inflammatory process is the activation of NF-kB.[46,48,49]

NF-kB is a potent proinflammatory gene transcription factor. When activated, it binds to specific genes, stimulating the production and release of the proinflammatory cytokines, namely TNF-α and IL-6, and adhesion molecules. These cytokines, when released in excess, favor the secretion of C-reactive protein by the liver.[35]

It is important to say that TRPM7 channels appear to regulate magnesium concentrations in tissues, and are therefore important in the homeostasis of this mineral.[50] Note, however, that TRPM7 is not selective for magnesium, and its expression may also mediate calcium influx and consequently inflammation, which depends on the serum concentrations of both minerals. That is, in situations of magnesium deficiency, calcium competes with magnesium and enters the cells.[51,52]

Magnesium deficiency appears to increase the production of free radicals and the sensitivity of cells to the attack of reactive oxygen species.[53,54] Hypomagnesemia favors the infiltration of neutrophils and macrophages in the affected cells, which potentiates the activity of the enzyme nicotinamide adenine dinucleotide phosphate oxidase

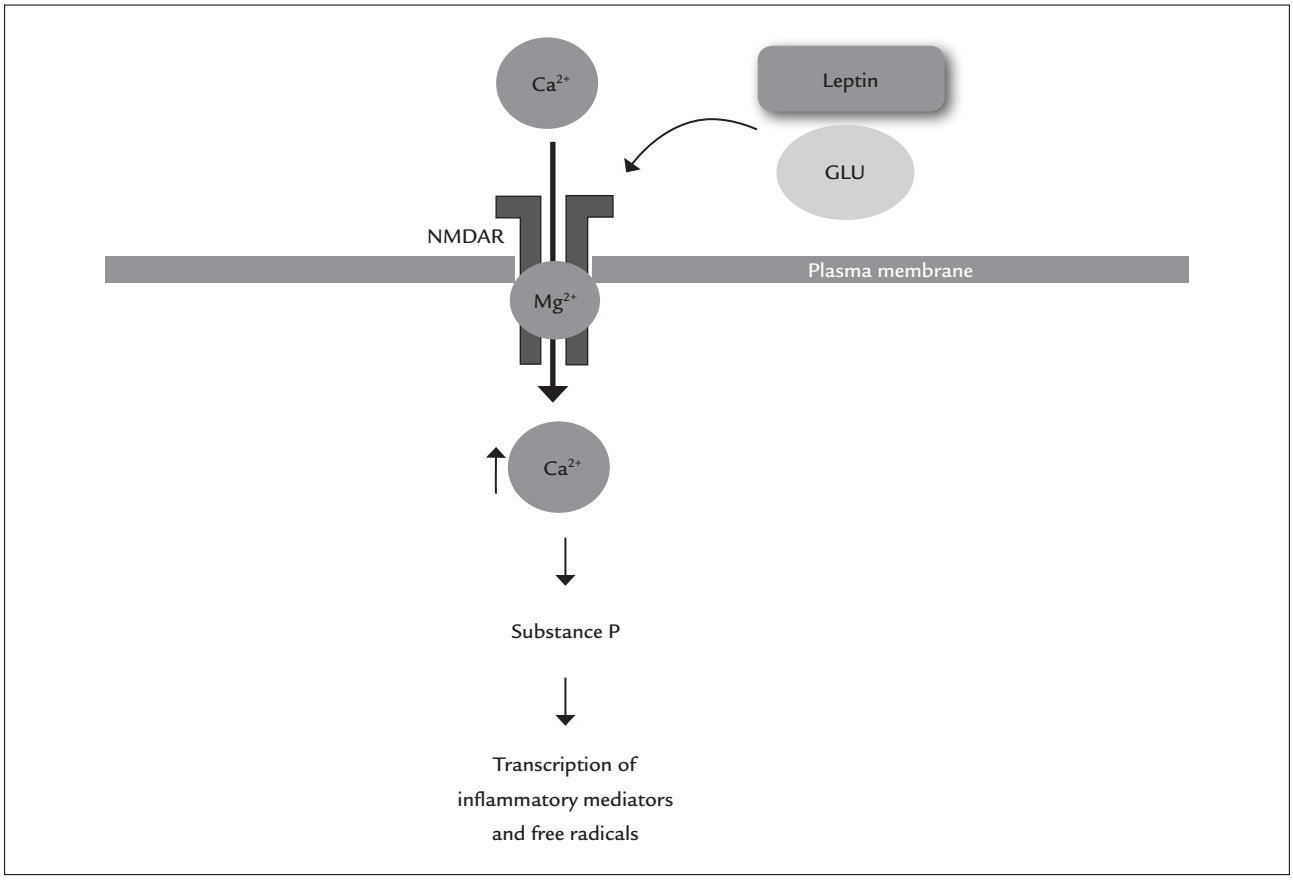

FIGURE 1 Action of magnesium as an anti-inflammatory nutrient in the brain. The increase of calcium in the intracellular medium promotes the transcription of inflammatory mediators through the release of substance P. Magnesium can inhibit this inflammatory pathway by its action as a natural calcium antagonist, blocking the increase of intracellular concentrations of this mineral.

Ca^{2+}: calcium; Mg^{2+}: magnesium; NMDAR: N-methyl-D-aspartate receptor; GLU: glutamate.

(NADPH oxidase), increasing the production of the superoxide radical.[46,55]

Hypomagnesemia also contributes to reduce the expression and activity of antioxidant enzymes, such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), and cellular and tissue antioxidant concentrations, as well as increases the production of hydrogen peroxide by inflammatory cells.[11,56]

Combined with this, in the presence of hypomagnesemia, intracellular ionic calcium contributes to the excessive production of uric acid and hydroxyl radical, which reacts with nitric oxide, which is also high in hypomagnesemia, forming peroxynitrite.[22,57] Thus, excessive production of reactive species in magnesium-deficient individuals also contributes to the inflammatory state present in obese individuals (Figure 2).

Some studies have been conducted to evaluate the effect of magnesium supplementation in obese or overweight individuals. However, no reduction in the concentration of inflammatory biomarkers was observed. Moslehi et al.[57] found that supplementation with 250 mg/day of magnesium oxide for 8 weeks was not able to reduce levels of C-reactive protein in overweight women. Rodriguez-Hernandez et al.[58] did not observe reduced concentrations of this inflammatory protein in obese women supplemented with 450 mg of magnesium chloride for 4 weeks, either.

Final considerations

Scientific evidence as presented in this review suggests that magnesium deficiency favors the manifestation of chronic low-grade inflammation in obese individuals. Nevertheless, mineral supplementation does not seem to influence the reduction of inflammatory biomarkers. Although some explanations have been proposed with a view to clarifying the role of the mineral in this disorder, the mechanisms are not yet fully identified. Therefore, new studies on the subject may provide biochemical

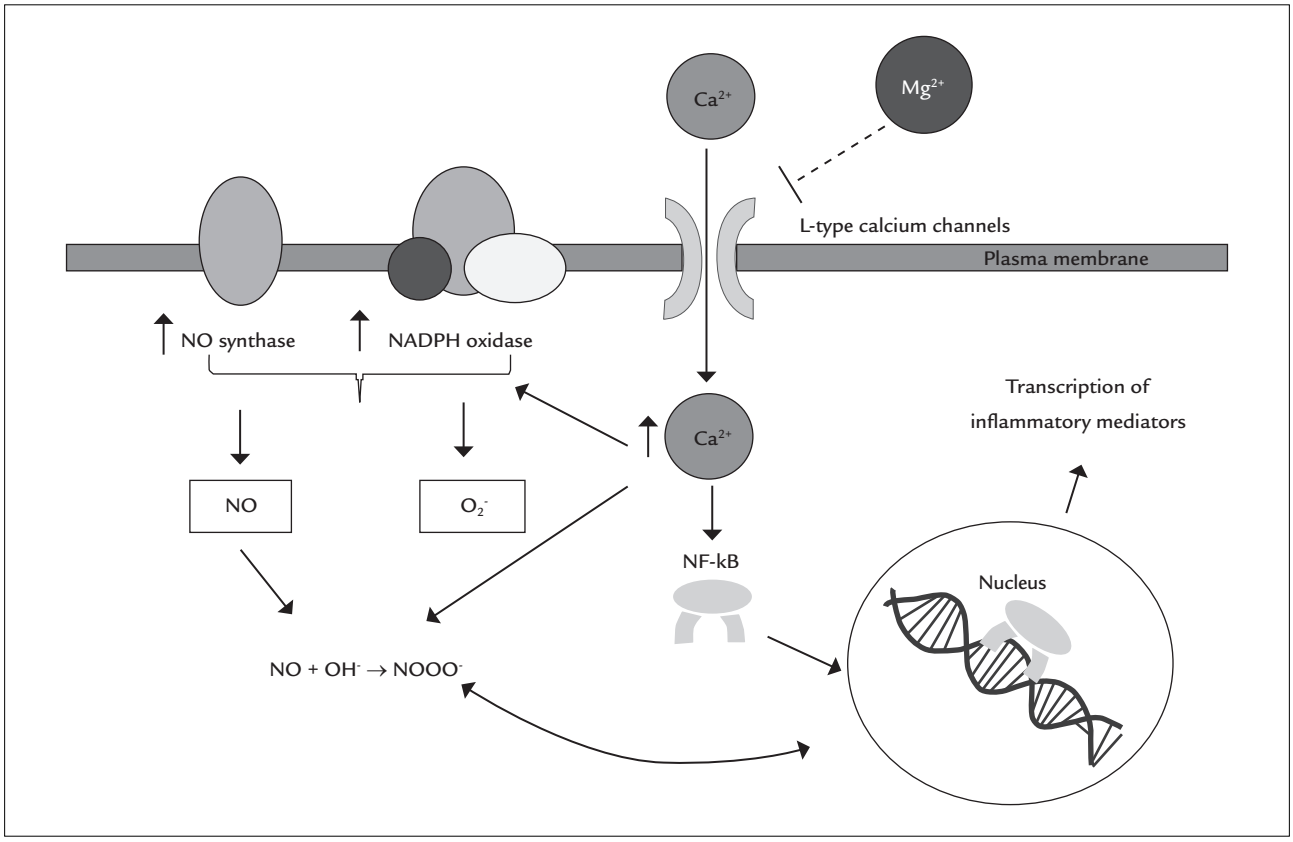

FIGURE 2 Action of magnesium as an anti-inflammatory nutrient in adipose tissue. The increase of calcium in the intracellular medium promotes the oxidation of cell membranes and the transcription of inflammatory mediators through the activation of NF-κB and its translocation into the nucleus, and increases the oxidative stress through the activation of the NO synthase and NADPH oxidase. Magnesium can inhibit this inflammatory pathway by its action as a natural calcium antagonist, blocking the increase of intracellular concentrations of this mineral.
Ca^{2+}: calcium; Mg^{2+}: magnesium; NF-κB: nuclear factor kappa B; NO synthase: nitric oxide synthase.

bases to explain the action of this nutrient as a protection against chronic inflammation present in obesity.

Conflict of interest

The authors declare no conflict of interest.

Resumo

Hipomagnesemia e sua relação com a inflamação crônica de baixo grau na obesidade

Introdução: O acúmulo de gordura visceral na obesidade está associado à produção excessiva de adipocinas pró-inflamatórias, o que contribui para o estado de inflamação crônica de baixo grau. A literatura também tem mostrado que a deficiência de minerais, em particular do magnésio, possui papel importante na patogênese desse distúrbio metabólico com repercussões clínicas relevantes.
Objetivo: Trazer informações atualizadas sobre a participação da hipomagnesemia na inflamação crônica de baixo grau em indivíduos obesos.
Método: Bases de dados Pubmed, SciELO, Lilacs e ScienceDirect, utilizando as palavras-chave: "obesity", "magnesium" e "low grade inflammation".
Resultados: As evidências científicas sugerem que a deficiência de magnésio favorece a manifestação da inflamação crônica de baixo grau em indivíduos obesos.
Conclusão: É evidente a participação do magnésio, por meio de reações bioquímicas e metabólicas, na proteção contra esse distúrbio metabólico presente na obesidade.

Palavras-chave: obesidade, magnésio, inflamação crônica de baixo grau.

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Systematic review of the synergist muscle ablation model for compensatory hypertrophy

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Article received: 2/21/2016
Accepted for publication: 6/26/2016

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http://dx.doi.org/10.1590/1806-9282.63.02.164

Summary

Objective: The aim was to evaluate the effectiveness of the experimental synergists muscle ablation model to promote muscle hypertrophy, determine the period of greatest hypertrophy and its influence on muscle fiber types and determine differences in bilateral and unilateral removal to reduce the number of animals used in this model.
Method: Following the application of the eligibility criteria for the mechanical overload of the plantar muscle in rats, nineteen papers were included in the review.
Results: The results reveal a greatest hypertrophy occurring between days 12 and 15, and based on the findings, synergist muscle ablation is an efficient model for achieving rapid hypertrophy and the contralateral limb can be used as there was no difference between unilateral and bilateral surgery, which reduces the number of animals used in this model.
Conclusion: This model differs from other overload models (exercise and training) regarding the characteristics involved in the hypertrophy process (acute) and result in a chronic muscle adaptation with selective regulation and modification of fast-twitch fibers in skeletal muscle. This is an efficient and rapid model for compensatory hypertrophy.

Keywords: ablation of synergists, compensatory hypertrophy, experimental models, muscle mass, skeletal muscle cross-sectional area.

Introduction

Skeletal muscle is highly adaptive and has a self-regulating capacity.[1-3] Hypertrophy is an example of this plasticity and refers to the increase in muscle mass necessary to enable the muscle to optimize its response to the demands of sustaining and generating force.[1,2,4-6]

Skeletal muscle mass is regulated by a variety of stimuli, the best known of which is mechanical overload. The muscle adaptation process can be induced by stretching/immobilization,[44,46] compensatory mechanisms (chronic).[1,2-6,8,9,12,14,18-20,61-65] and exercise/training.[33,46] Evidence of this is derived from a large number of studies demonstrating that overload leads to an increase in muscle mass and cross-sectional area of the muscle fibers and induces chronic changes in the balance between the synthesis and degradation of proteins.[2,7-9] Compensatory hypertrophy through the ablation of synergists of plantar flexion is one of the ways to produce chronic overload experimentally.[3,7,12,13,20,59] The ablation of synergists for compensatory hypertrophy consists of the surgical removal of all or part of synergistic muscles, which can be either unilateral or bilateral, to generate chronic functional overload that causes hypertrophy.[3,7,12,13,20,59] According to Parvaresh et al.,[1] complete muscle removal can compromise the neurovascular supply, which increases edema and the recovery of the animal in the postoperative period. Thus, the removal of only the distal portion of synergist muscle is recommended (Figure 1).

The synergist muscle ablation model induces muscle hypertrophy in only a few days, thereby facilitating the study of adaptive responses.[2,3,7,10-20] The most studied muscles are plantar flexors in the rear paw of rats. As

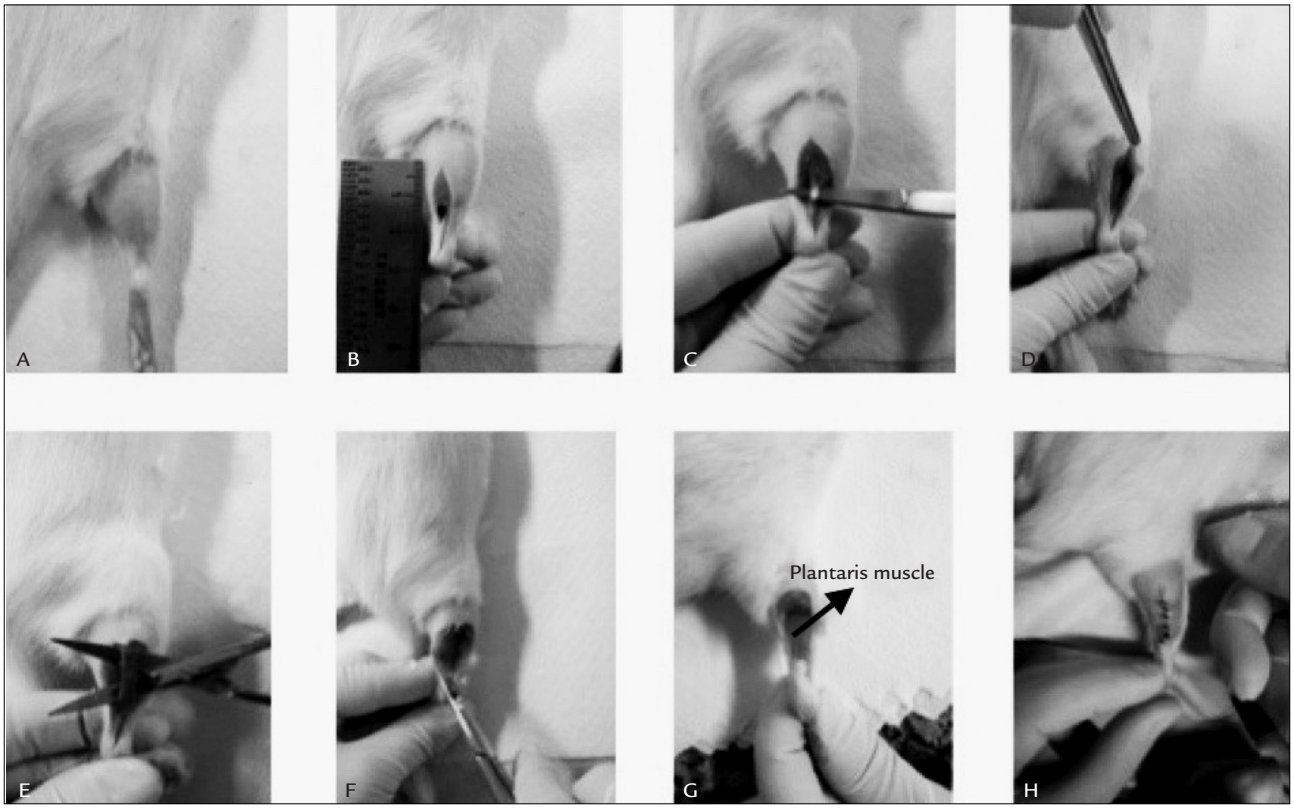

FIGURE 1 Synergist ablation surgery of plantaris muscle. A. Shaving the back of the hind leg. B. Incision of 2 cm. C. Tendon of gastrocnemius muscle. D. Partial removal of the lateral gastrocnemius muscle. E. Soleus muscle – total removal. F. Partial removal of the medial gastrocnemius muscle. G. The plantaris muscle is isolated. H. Suture with seven points.

skeletal muscle has different types of fibers (type I [slow-twitch] and type II [fast-twitch – IIa, IIb, IIx/IId]),[21] a number of authors justify the choice of the plantaris muscle due to its diversity of fiber types (type I: 8 ± 2%; type IIA: 19 ± 3%; type IIB/D: 74 ± 4%) and its different adaptation possibilities.[9] Compensatory hypertrophy induced by the functional elimination of synergistic muscles results in an increase in muscle fiber diameter and muscle mass as well as the regulation of protein synthesis in different types of muscle fibers.

The present systematic review of the literature discusses the results found in studies using this experimental model to cause overload in the plantar muscle of rats, comparing the findings with regard to the percentage increase in the mass of the plantar muscle, the period of greatest muscle mass gain and differences between unilateral and bilateral surgery. The aim of this review was to evaluate the effectiveness of the experimental synergist muscle ablation model to promote muscle hypertrophy in different overload models, to determine the period of greatest hypertrophy and its influence on muscle fiber types, and to determine differences in bilateral and unilateral removal to reduce the number of animals used in this model, thereby facilitating its reproduction and its choice among different chronic hypertrophy models.

Method

The methods were based on PRISMA guidelines. Searches were performed in the PubMed, ScienceDirect, MEDLINE and CAPES Portal databases for articles published between January 1999 and July 2013 using the keywords "compensatory hypertrophy" AND "mechanical overload" OR "ablation of synergists" AND "compensatory hypertrophy" AND "experimental models" OR "skeletal muscle cross-sectional area." The following criteria were used for the selection of papers: (1) the use of a rat model; (2) the use of synergist ablation to overload the plantaris muscle; (3) bilateral or unilateral muscle removal; and (4) determination of the cross-sectional area of muscle fibers or muscle mass. Review articles were excluded, as well as other experimental models and in vitro studies. Articles that used overload in another muscle and did not report on their studies the cross-sectional area (CSA) or muscle mass were also excluded.

A total of 63 articles were retrieved using combinations of the keywords. Twenty-four papers were review articles;[7,9,21-42]

eight studies used a model other than the ablation of synergists to cause hypertrophy;[43-50] and six were in vitro studies.[51-56] All these studies were excluded. Among the remaining 25 studies, seven did not compare the cross-sectional area of the muscle and/or muscle mass to a control group and were excluded.[3,13,15,48,57,59,60] Thus, 19 studies met the inclusion criteria and were selected for the present review (Figure 2).

Statistical analysis

The data from graphs were grouped based on collection time and percentage of increase in mass of the plantaris muscle with error propagation. A scatter plot was created to show the distribution of muscle mass gain in function of the number of days following the ablation procedure. Two regressions were employed: one for less than 15 days of data and another for more than 15 days of data. A slope of the regression line coefficient of 0.042 ± 0.002% and linear coefficient of 0.095 ± 0.021% was used for this calculation (slope of the regression line coefficient + x linear coefficient, in which x is the number of days). R^2 values demonstrate how the data approaches the progression and form a straight line (R^2 = 0.52 in the first 15 days following the ablation of synergist muscles and R^2 = 0.06, 15 days after surgery). Values greater than 50% demonstrate that the linear fit is adequate. Chebyshev's inequality test was used to compare muscle mass following unilateral or bilateral removal. This test makes no assumptions regarding the normality of the data distribution and only requires the means and standard errors as inputs. Only periods of 14 and 28 days were compared, which were the periods used by most authors. The results were p=0.2996 for 14 days and p=0.2584 for 28 days.

Results

Table 1 summarizes the findings of the 19 articles analyzed in the present systematic review. Considerable variation was found in the analysis period following the ablation of synergists. Increases in muscle mass (g) and

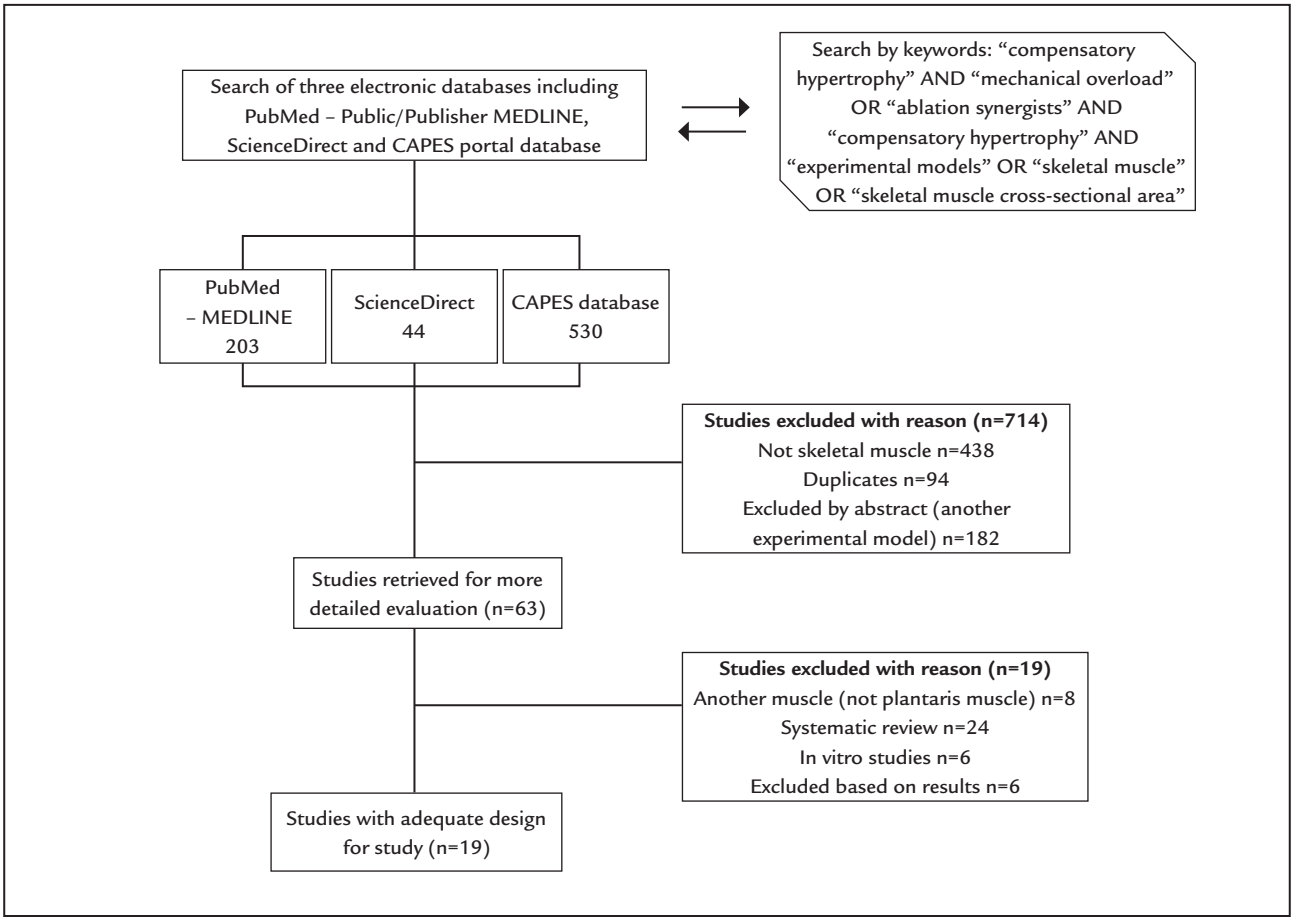

FIGURE 2 Flowchart of the selection process of literature according to the PRISMA guidelines.

fiber cross-sectional area (μm^2) of the plantar muscle were reported in all studies evaluated, demonstrating compensatory hypertrophy.

The data were grouped based on collection time and percentage of increase in mass of the plantar muscle with error propagation. The trend line revealed linear progression up to 15 days, with stabilization of the data after this period. The method of least squares was used, including the error of the data reported by the authors. For studies that did not provide such information, the mean error was used. The trend line in Figure 3 shows the percentage (± error) of increase in muscle mass according to days after surgery as follows: 13.6 ± 2.1% one day after ablation, 38.7 ± 2.6% seven days after ablation and 68.0 ± 3.6% 14 days after ablation.

Only periods of 14 and 28 days were compared, which were the periods used by most authors. Both groups presented a large effect size (1.15 and 1.39 for 14 and 28 days, respectively) but since the authors made no assumptions regarding the data's distribution, the p-values were higher than the significance level (p=0.2996 for 14 days and p=0.2584 for 28 days), thus no significant differences were found between unilateral and bilateral surgery in the two periods. At 28 days, there is an overlap between both 95% confidence intervals ([68%, 85%] and [45%, 61%] for unilateral and bilateral, respectively) but no overlap was found at 14 days ([38%, 55%] and [45%, 61%] for unilateral and bilateral, respectively). By not assuming the normality of the data's distribution, the authors guarantee the probability of the type I error at $\alpha = 0.05$ at the expenses of an increased probability of the type II error. Therefore, despite the lack of statistical significance, the power of the test was low due to the limited data in the literature on unilateral synergist ablation reporting the percentage gain in plantaris muscle mass.

All data were grouped based on the period after the ablation of synergists (Figure 4). Greatest hypertrophy occurred between 12 and 15 days postoperatively. The increase in the cross-sectional area of the muscle and muscle fibers was studied using histological techniques.[1,7-9,60] The mean increase in cross-sectional area in comparison to the control was 66 ± 4% at day 14, demonstrating that compensatory hypertrophy is an effective model for increasing muscle mass.

Discussion

Compensatory hypertrophy occurs in response to a sustained increase in the mechanical load of skeletal muscle. Although the mechanisms involved in compensatory hypertrophy are not yet fully understood, this is an intense topic of research, which includes the definition, measuring, loading stimulus parameters, acute responses, hyperplasia, experimental models, adaptations of muscle fiber types, the involvement of satellite cells and endocrinology. The purpose of the present systematic review was to gather results reported by researchers who have used the standard ablation of synergists (gastrocnemius and soleus muscles) model to determine the induction of hypertrophy in the plantar muscle of rats, comparing the percentage of muscle gain to facilitate and standardize the use of this model for the study of muscle plasticity following functional overload. Increasing interest in the molecular and cellular mechanisms responsible for hypertrophy in recent years[1,6-9] underscores the need for a reliable and easily reproducible model. Rats are often used due to their considerable activity and their larger size in comparison to mice. The mean weight of the animals used in the studies analyzed was 220 ± 12 g.[1-5,7,14-16,18]

Among the models described in the literature for changes in muscle demand, different protocols of mechanical loading have been used: resistance training (RT) and compensatory hypertrophy after ablation and tenotomy.[2,3,5-7,13,18,65] Current theories suggest differences between mechanisms that induce hypertrophy through exercise and compensatory hypertrophy. Both methods cause changes in the muscle, but the molecular signaling pathways seem to be different.[26,49] Compensatory hypertrophy due to the ablation of synergists and tenotomy differ in terms of phases. The former has two distinct phases: an inflammatory phase, followed by the response of the muscle to the demand for a functional increase. Tenotomy has the disadvantage of the rapid reconnection of the cut tendon, which limits functionality.[65]

Most commonly studied muscles

Compensatory hypertrophy by the ablation of synergists is an efficient model for studies on muscle hypertrophy,[2,3,7,10-20] as the fast increase in muscle mass reduces the duration of the experiment. In recent years, changes have occurred in the standard of surgery (bilateral or unilateral) and the relationship between the number of days and increase in muscle mass, which justifies this systematic review.

In rats, the most commonly studied muscles are the tibial anterior, digitorum longus, soleus and plantar muscles.[1-5,7,14-16,18] In the 1980s and 1990s, the model most often employed was the entire removal of the tibialis anterior to generate overload of the digitorum longus.[15] Currently, the most used muscles in such models are the soleus and plantar muscle and compensatory hypertrophy commonly involves the removal of the distal portion of

TABLE 1 Studies selected for review using the synergist ablation model for compensatory hypertrophy.

Article	Ablation of synergists	Study design	Data collection	Outcomes (compared to control)
Adams et al.[4]	Unilateral	Plantar mass Rat body mass	6, 12, 24 and 48 h 3, 7 and 12 days	**12 d** Body mass (g) (226 ± 5 to 257 ± 6) Muscle mass (mg/g) (1.07 ± 0.02 to 1.73 ± 0.18)
Dunn et al.[64]	Bilateral	Cross-sectional area of muscle	7, 14 and 28 days	**28 d** Cross-sectional area increased 75% compared to control
Bodine et al[5]	Bilateral	Muscle mass	7, 14 and 30 days	**7 d** Muscle mass increased by 25% **14 d** Muscle mass increased by 38%
Adams et al.[65]	Bilateral	Cross-sectional area	6 and 24 h 3, 7, 15 and 90 days	**90 d** 46% increase in cross-sectional area of muscle fibers
Lee et al.[62]	Bilateral	Muscle mass	1, 3, 7 and 21 days	**1 d** Increased by 10% **3 d** Increased by 31% **21 d** Increased by 21%
Yamaguchi et al.[12]	Unilateral	Cross-sectional area	3 and 14 days	**14 d** Increased by 43.3 ± 3.8%
Sakuma et al.[63]	Unilateral	Muscle mass	1, 2, 3, 4, 6, 8, 10, 14 and 28 days	**1 d** Increased by 56% **3 d** Increased by 40,9% **6 d** Increased by 31.3 % **10 d** Increased by 44.8 % **14 d** Increased by 46.8% **28 d** Increased by 76.2%
Pehme et al.[14]	Bilateral	Muscle mass	14 days	**14 d** Increased by 40%
DiPasquale et al.[1]	Bilateral	Cross-sectional area	1, 3, 5 and 14 days	**3 d** Increase in peak edema No statistical difference in cross-sectional area of muscle in 3 days
Marino et al.[6]	Bilateral	Cross-sectional area Peak edema	3, 7 and 14 days	**3 d** Statistical difference in cross-sectional area of muscle Retention of 90% water **7 d** 5% Increase in cross-sectional area of muscle Retention of 70% water **14 d** 21% increase in cross-sectional area of muscle Retention of 45% water
Novack et al.[18]	Bilateral	Plantar mass Peak edema	1, 3, 5 and 14 days	**14 d** 80% increase in muscle mass Peak edema in 5 days
Huey et al.[19]	Bilateral	Relative and absolute plantaris muscle	12 h, 1, 2, 3 and 7 days	**7 d** Relative mass increased by 15% Absolute mass increased by 21%
Pavaresh et al.[2]	Bilateral	Absolute and relative mass	3 and 7 days	**3 d** Absolute mass increased by 10% Relative mass increased by 18% **7 d** Absolute mass increased by 21% Relative mass increased by 20%
Goodman et al.[7]	Bilateral	Cross-sectional area	7 and 14 days	**14 d** Increased by 30%
Schuenke et al.[9]	Bilateral	Cross-sectional area	28 days	**28 d** Increased by 35% in young rats Increased by 21% in older rats
Goodman et al.[8]	Bilateral	Cross-sectional area	10 days	**10 d** 1,000 ± 60 vs. 2,000 ± 200 (μm^2)
Gordon et al.[20]	Bilateral	Muscle mass	1 and 3 days	**1 d** Increased by 48 ± 9% (m±SD) **3 d** Increased by 73 ± 17% (m±SD)
Bentzinger et al.[61]	Unilateral	Muscle mass	7 and 28 days	**7 d** Increased by 90% **28 d** Increased by 120%

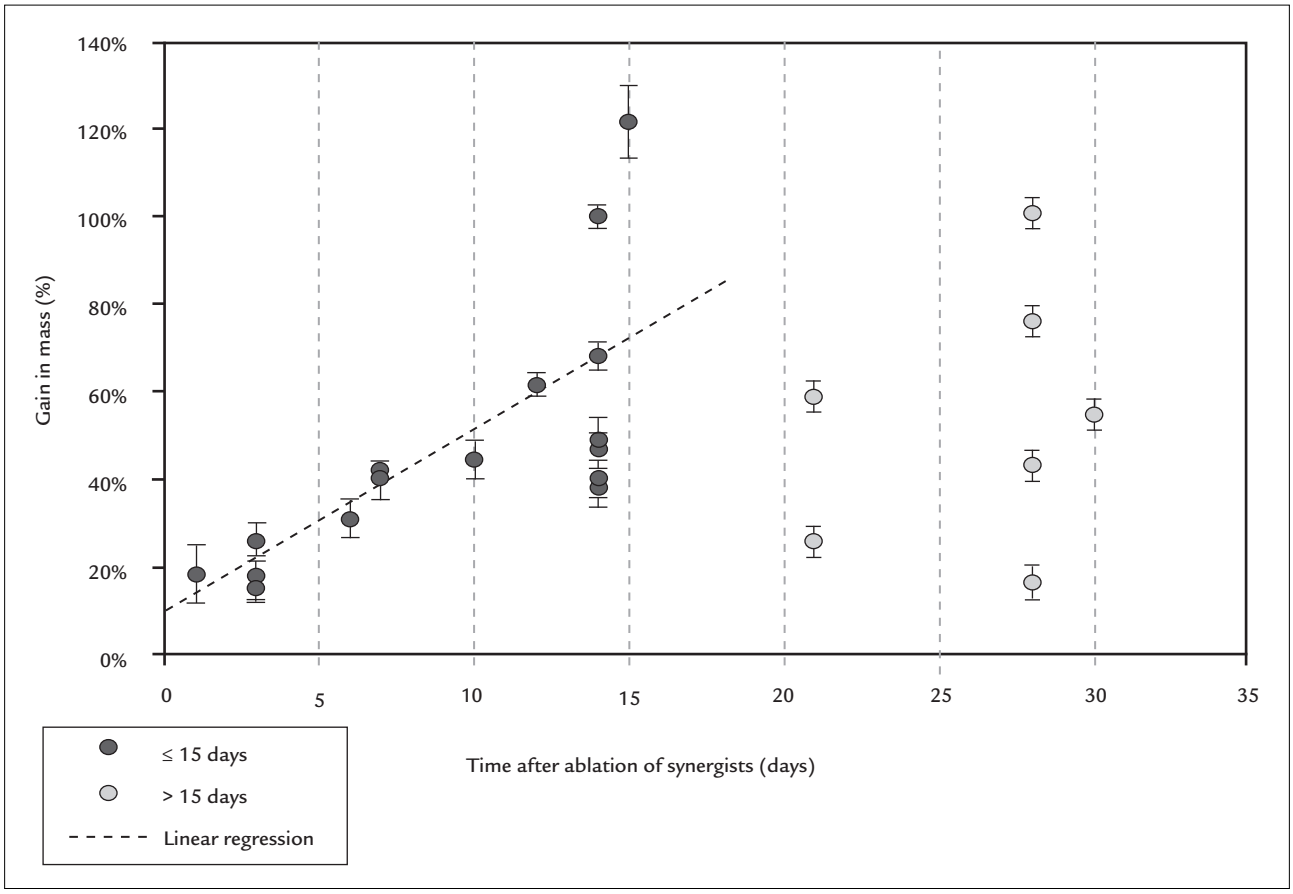

FIGURE 3 Distribution of plantaris muscle hypertrophy according to number of days after ablation of synergists. Dark gray dots represent data collected in less than 15 days after ablation. Light gray dots represent data collected 15 days after ablation of synergists. The trend line demonstrates linear progression up to 15 days, with stabilization thereafter.

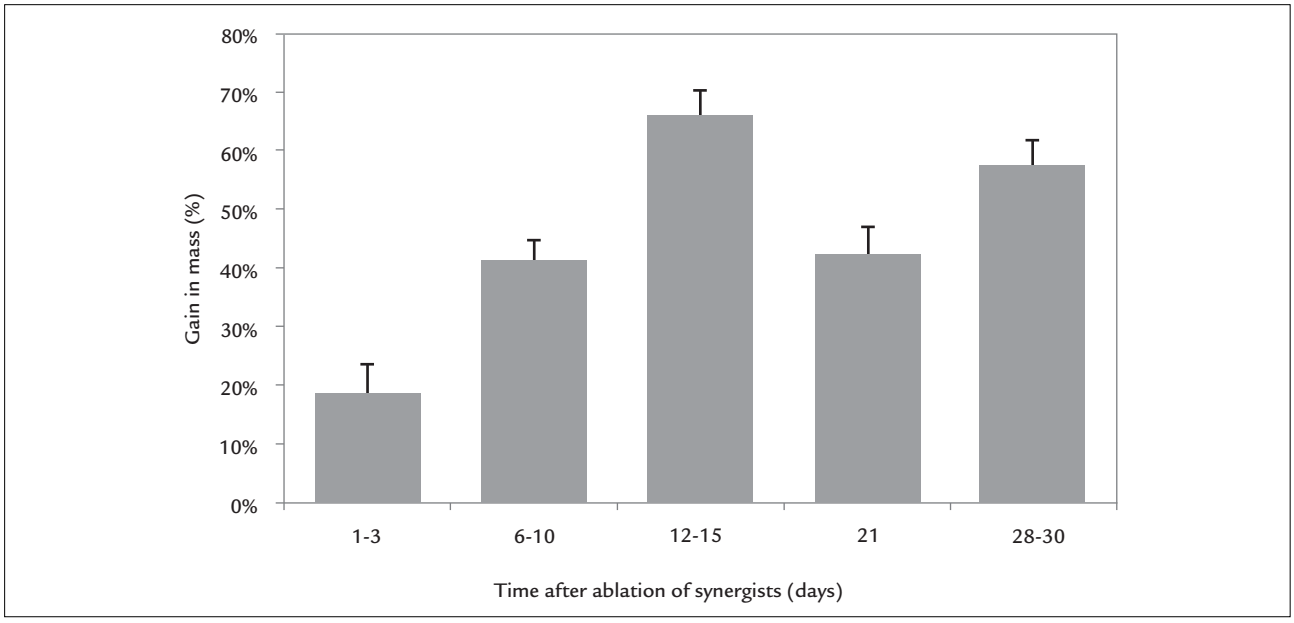

FIGURE 4 Mean gain in muscle mass according to time after the ablation of synergists based on the literature.

the gastrocnemius. Despite displaying anatomical proximity in rats, these muscles are distinct in their architecture and biochemistry.[6,9,16,19,58]

Considering the different types of muscle fiber (slow-twitch [type I] and fast-twitch [type IIa, IIb, IIx and IId]),[21] a number of authors justify the choice of the plantar muscle for studies on adaptation due to its composition of different fiber types. The plantar muscle is predominantly composed of fibers IId and therefore has a smaller amount of mitochondria as such fibers use the glycolytic pathway for a faster response during gait. Authors attribute this adaptation feature of the plantar muscle to its constant activation during the stance phase and weight bearing in quadrupeds, which use this muscle to resume ambulation.[9]

Unilateral vs. bilateral surgery

Based on the data analyzed, both unilateral and bilateral synergist ablation lead to an increase in muscle mass, with no statistically significant difference between the two types of surgery. Researchers working with unilateral surgery report a mean increase in muscle mass of 46.8 ± 2.6% 14 days following synergist ablation,[4,12,61,63] whereas those working with bilateral surgery report an increase of 52.3 ± 3.1% in the same period.[4,12,61,63] Although the number of studies involving unilateral ablation (n=4) was smaller than the number involving bilateral ablation (n=14), the similar increase in muscle mass demonstrates the benefits of unilateral surgery, which reduces the number of animals used in experiments and is in line with the goals of the International Council for Laboratory Animal Sciences.

Expected time for hypertrophy

The data collection period varied considerably among the studies analyzed. Moreover, it is important to determine how the data are distributed for adequate visualization of the period of greatest hypertrophy. The synergist ablation model led to an increase in muscle mass in the first three days due to inflammation and edema caused by the surgical procedure.[1,2,6,20,62,63] This disadvantage in the compensatory hypertrophy model by synergist ablation is due to the inflammation process that occurs after surgery. However, Novack et al.[18] demonstrated that components of the acute inflammatory response are required in the muscle repair and remodeling process and the intensity of the inflammatory response is related to the magnitude of hypertrophy. With synergist ablation, the increase in prostaglandin-endoperoxide synthase 2 (COX-2) seems to be related to the considerable increase in muscle mass that occurs in this model and the inflammatory response enables and facilitates the activity of extracellular proteases, the accumulation of macrophages and cell proliferation, including the activation and proliferation of satellite cells, which seems to exert an influence on the greater hypertrophy achieved with this model in comparison to exercise-induced hypertrophy.

According to Marino et al.,[6] no statistically significant difference in the cross-sectional area of the muscle fibers was found in the first three days following ablation. At 3 to 5 days, the edema is reduced, followed by an increase in the cross-sectional area of the muscle fibers as well as enzyme activity and protein synthesis, which constitute hypertrophy as an adaptation to the new condition of chronic overload.[1,6,18,61,63] The period of 12 to 15 days was identified as that with the greatest percentage increase in muscle mass in comparison to the control (Figure 3), demonstrating a linear progression (i.e., a progressive gain in muscle mass over the first 15 days after ablation). At 28 days, the authors found no further increase in gene expression related to increased muscle mass,[9,64,65] as demonstrated by the cessation of linear progression and stabilization of the data (Figure 2). Thus, peak hypertrophy (greatest increase in muscle mass and cross-sectional area of the muscle fibers) occurs between the second and third week following synergist ablation. Concentrating studies on this period is fundamental to determining the impact of novel therapies and interventions designed either to diminish or potentiate the effects of compensatory muscle hypertrophy.

Cross-sectional area and types of muscle fiber

The increase in the cross-sectional area of the muscle and muscle fibers was studied using histological techniques.[1,7-9,61] The mean increase in cross-sectional area in comparison to the control was 18.66% in 14 days, demonstrating that compensatory hypertrophy is an effective model for increasing muscle mass. The trend line in Figure 2 shows the percentage increase in muscle mass according to days following surgery: approximately 10% one day after ablation, 38% seven days after ablation and 68% 14 days after ablation.

The increase in the cross-sectional area of muscle is related to protein synthesis of the muscle fibers and the activation of satellite cells. Studies suggest that satellite cells are responsible for both the growth of muscle fibers and the regulation of the muscle fiber phenotype.[8,14,19,20,37,46] At the onset of compensatory hypertrophy, the muscle fiber alters its response. The relationship among the cross-sectional area, hypertrophy and fiber type[37] indicates that chronic overload induces changes in the expression of heavy chain myosin.

Goodman et al.[8] demonstrated a significant increase in protein synthesis in four types of muscle fiber (slow-twitch [type I] and fast-twitch [type IIa, IIb and IIx]) in the plantaris muscle in rats submitted to synergist ablation. Type IIb fibers exhibited the least amount of protein synthesis, whereas IIa fibers exhibited the most amount of protein synthesis, which did not differ significantly from that found in type I fibers. In the cross-sectional area, type IIb fibers were shorter than IIa fibers, which also exceeded the area found in type I fibers. These findings suggest that this model results in the selective regulation and modification of fast-twitch fibers in skeletal muscle.

Conclusion

Based on the findings of the present systematic review, the following conclusions may be drawn: 1. the synergist ablation model differs from other overload models regarding the characteristics involved in the hypertrophy process; 2. 12 to 15 days following ablation is the period of greatest muscle hypertrophy; 3. the lack of a significant difference in the gain in muscle mass between unilateral and bilateral ablation demonstrates that contralateral limb can be used as the control, which reduces the number of animals used in this model; and 4. synergist muscle ablation is an efficient reproducible model for achieving rapid hypertrophy and results in the selective regulation and modification of fast-twitch fibers in skeletal muscle.

Acknowledgment

Funding for this study was provided by Universidade Nove de Julho, São Paulo, Brazil.

Conflict of interest

The authors declare no conflict of interest.

Resumo

Revisão sistemática do modelo de ablação dos músculos sinérgicos na hipertrofia compensatória

Objetivo: Avaliar a eficácia do modelo experimental de ablação dos sinergistas para promover a hipertrofia muscular, determinar o período de maior hipertrofia, sua influência sobre os tipos de fibras musculares e determinar diferenças na remoção unilateral ou bilateral para reduzir o número de animais utilizados nesse modelo.
Método: Após a aplicação dos critérios de elegibilidade para sobrecarga mecânica do músculo plantar em ratos, 19 artigos foram incluídos na revisão.
Resultados: Ocorre maior hipertrofia entre os dias 12 e 15, o que torna o modelo eficiente para alcançar a hipertrofia rapidamente. O membro contralateral também pode ser usado, pois não houve diferença entre a cirurgia unilateral e bilateral, o que reduz o número de animais usados no experimento.
Conclusão: O modelo difere de outros modelos de sobrecarga (exercício e treinamento) em razão das características envolvidas no processo de sobrecarga imposta (aguda), resultando em uma adaptação crônica muscular com modificação de fibras de contração rápida do músculo esquelético. É um modelo rápido e eficiente para se estudar hipertrofia compensatória.

Palavras-chave: ablação dos sinergistas, hipertrofia compensatória, modelos experimentais, massa muscular, área de secção transversa do músculo esquelético.

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Osteoporosis and autophagy: What is the relationship?

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Study conducted at Universidade Federal de São Paulo – Escola Paulista de Medicina (Unifesp-EPM); Faculdade de Odontologia da Universidade Estadual Paulista (Unesp); Faculdade de Medicina da Universidade de São Paulo (FMUSP), SP, Brazil

Article received: 5/10/2016
Accepted for publication: 5/31/2016

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http://dx.doi.org/10.1590/1806-9282.63.02.173

Summary

Autophagy is a survival pathway wherein non-functional proteins and organelles are degraded in lysosomes for recycling and energy production. Therefore, autophagy is fundamental for the maintenance of cell viability, acting as a quality control process that prevents the accumulation of unnecessary structures and oxidative stress. Increasing evidence has shown that autophagy dysfunction is related to several pathologies including neurodegenerative diseases and cancer. Moreover, recent studies have shown that autophagy plays an important role for the maintenance of bone homeostasis. For instance, in vitro and animal and human studies indicate that autophagy dysfunction in bone cells is associated with the onset of bone diseases such as osteoporosis. This review had the purpose of discussing the issue to confirm whether a relationship between autophagy dysfunction and osteoporosis exits.

Keywords: autophagy, bone tissue, osteoblast, osteocyte, osteoclast, osteoporosis.

Introduction

Bone tissue is constantly being remodeled by the coordinated action of osteoclasts, osteoblasts, osteocytes, and bone lining cells.[1,2] Osteoclasts and osteoblasts are respectively responsible for bone resorption and formation, while osteocytes are important in maintaining and controlling bone remodeling.[2-5] Bone remodeling is necessary for the repair of microfractures, as well as for skeletal adaptation to different mechanical stimuli and for calcium homeostasis.[6] However, any imbalance in this process so that bone reabsorption exceeds formation can result in bone loss and subsequent osteoporosis.[7-10]

Osteoporosis is a systemic bone disease characterized by progressive loss of bone mass, bone fragility and susceptibility to fractures.[11] The incidence of osteoporotic fractures, associated with morbidity, mortality and the costs of these fractures, has increased substantially and became a public health problem in many countries,[12-14] including Brazil.[15-17] As life expectancy increases, the number of osteoporotic fractures is expected to increase from 1.7 million in 1990 to 6.3 million in 2050.[18]

The cause of osteoporosis is multifactorial and includes genetic, hormonal and nutritional factors, combined with people's lifestyle choices.[19,20] Peak bone mass is reached early in adult life and from this point both men and women start to lose bone mass more or less depending on a combination of intrinsic and extrinsic factors.[21,22] This process can be aggravated by a lack of physical exercise,[23] prolonged treatment with corticoids,[24] estrogen deficiency, especially in postmenopausal women,[25] presence of other chronic diseases, and by aging.[20]

Recent evidence has pointed out that autophagy, a cell survival pathway, plays an important role in the maintenance of bone homeostasis,[26-28] and changes in this pathway have been related to osteoporosis.[29,30] Since osteoporosis became a serious public health problem with an incidence rate that is likely to increase in the next decades, understanding the cellular and molecular mechanisms involved in the process of bone loss is crucial and paramount for the development of new therapies.

In this review, we discuss studies that associate the autophagic pathway with osteoporosis, aiming to better

understand if there is a possible correlation between these two processes.

Autophagy

Autophagy (from the Greek *autos*, self, and *phagein*, eat) is a lysosomal degradation pathway responsible for the degradation and recycling of cellular components such as unnecessary organelles and proteins, also serving to destroy intracellular pathogens.[31-33] Autophagy occurs in every cell so that structures that are unnecessary to the cell can be recycled. However, factors that induce cellular stress, such as hypoxia, caloric restriction and the accumulation of oxidative stress, can induce autophagy.[34-36]

Three types of autophagy are described: macroautophagy, microautophagy and chaperone-mediated autophagy. The latter involves direct translocation of cytoplasmic proteins into lysosomes, aided and directed by chaperone proteins.[37] In microautophagy, invagination of the lysosomal membrane results in small portions of the cytoplasm being captured towards the lysosome lumen.[38,39] Macroautophagy, hereafter referred to as autophagy, is the most studied of the three autophagic pathways, it has been maintained throughout evolution, from yeast to mammals, and is characterized by the formation of autophagosomes.[40,41] After autophagic stimulation, a small vesicle called the phagophore elongates and subsequently surrounds a portion of the cytoplasm, resulting in the formation of a double membrane structure, termed autophagosome.[31,42] The membrane of the autophagosome then fuses with a lysosome, resulting in the degradation of the entrapped material. Amino acids, fatty acids and other molecules resulting from degradation return to the cytoplasm for recycling and energy production.[38,42]

Usually, the autophagic process is divided into four main steps: initiation/nucleation, elongation, maturation and degradation.[38,42] During initiation, which takes place after autophagic stimulus, a membrane known as phagophore is formed. Phagophore formation and nucleation occur with the formation of two multiprotein complexes: one is composed of proteins ULK1/2, Atg13 and FIP200, and the other, which is known as class III phosphatidylinositol 3-kinase (PI3K) complex, is formed by proteins beclin-1, Vps15, Vps34, Ambra1, UVRAG, and more.[33,43] These complexes, with the participation of several other proteins, are responsible for recruiting and initiating phagophore elongation.[44] Beclin-1 is fundamental for the formation of PI3K complexes and, therefore, has been commonly used as a marker of autophagia.[45]

After nucleation, phagophore elongation occurs and an autophagosome is formed. This process is coordinated by two protein complexes called ubiquitin-like conjugation system, formed by several Atgs (autophagy-related genes) proteins, including Atg3, Atg4, Atg5, Atg7, Atg10, Atg12 and Atg16. This system facilitates conjugation of a molecule called LC3 (a light chain of the microtubule-associated protein 1) with phospholipid phosphatidylethanolamine to LC3II. LC3II then enters the autophagosome's membrane, assisting its elongation and closure.[38,46] Therefore, it is currently known that LC3II is found within the autophagosome's membrane, being key to its formation. Because of that, LC3II has been widely used as a specific autophagosome marker.[38,47]

The other stages of autophagy consist in autophagosome maturation and degradation of its contents. Autophagosome maturation refers to its fusion with components of the endocytic pathway, such as early and late endosomes and lysosomes, turning the contents inside the autophagosome acid.[48] Several proteins involved in vesicular transport, such as dynein, and membrane fusion in the endocytic pathway, including Rab7, SNARES and ESCRT, beclin-1, Vps34 and UVRAG, are involved in autophagosome maturation.[49] The last stage of the autophagic pathway is that of degradation of the autophagosome's contents after fusing with a lysosome and subsequent reuse of the resulting molecules (lipids, amino acids, nucleotides, etc.) as a manner of recycling cell components and producing energy.[50]

For a long time, autophagy was considered a pathway of non-selective degradation.[48] However, it is currently accepted that this process can be extremely specific since organelles are selectively targeted for degradation.[51] The most widely known example of protein-specific autophagic degradation is that of protein p62 (ou SQSTM1), which binds to ubiquitinated proteins leading them to the interior of autophagosomes.[52] This mechanism is mediated by binding of p62 to LC3II present in the autophagosome's membrane, so that p62 is also internalized and ultimately degraded. This protein is thus considered as one of the main substrates for autophagosomes and, therefore, its increased expression indicates decline in the autophagic process and vice versa. Analyses of the combined expression of proteins p62 and LC3II are commonly used to assess the autophagic flow in cells.[53,54] A summary of the major stages of the autophagic pathway is illustrated in Figure 1.

Role of autophagy in bone biology

In recent years, a growing number of studies has shown that autophagy plays an important role to maintain bone homeostasis.[26,27,55] For example, cultures of autophagy

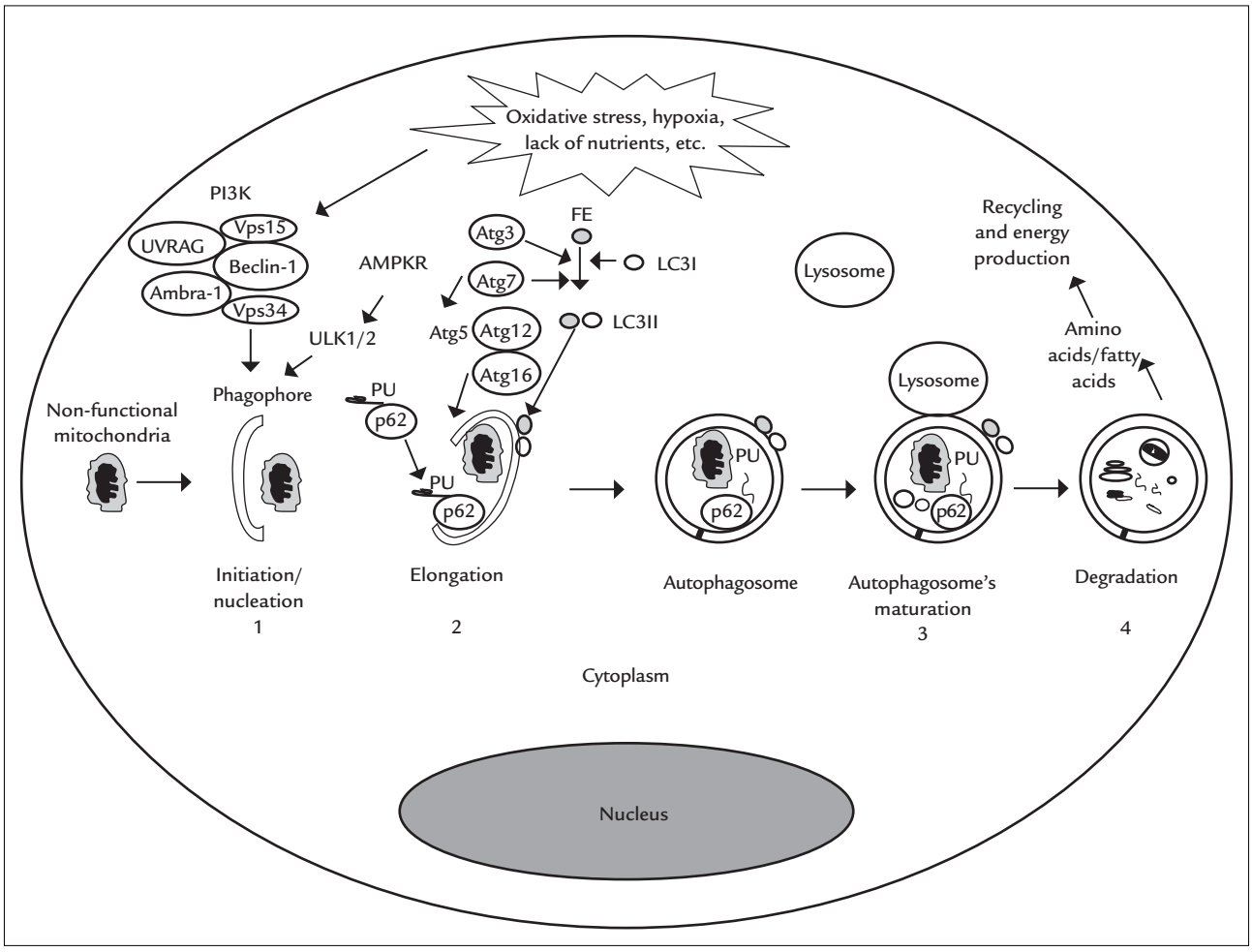

FIGURE 1 Schematic model of the four main stages of the autophagic pathway: 1. initiation/nucleation; 2. elongation; 3. maturation; and 4. degradation. After autophagic stimulation (oxidative stress, hypoxia, lack of nutrients, etc.), phagosome formation/nucleation is observed (1), assisted by multiprotein complexes PI3K (formed by beclin-1, Vps34/15, Ambra-1 and UVRAG) and ULK1/ULK2, as well as Atgs. Assisted by protein LC3II, the phagophore elongates and circles organelles and proteins that are no longer functional (2), including polyubiquitinated proteins (PU) carried by p62, forming the autophagosome. The latter may fuse with a lysosome (3), so that its contents are degraded (4).

gene-knockout osteoblasts (*BECN-1, ATG7* e *LC3*) result in deficiency in the process of mineralization.[56]

In vitro studies show that pharmacological inhibition of autophagy in osteoblast-like cells increases oxidative stress and stimulates apoptosis in these cells. In contrast, induction of autophagy in cultures of osteoblast-like cells reduces their oxidative stress and inhibits apoptosis.[57] It has also been reported that estrogen inhibits osteoblast apoptosis in vitro, inducing autophagy in these cells.[58]

Autophagy is also essential when osteoblasts are incorporated into the bone matrix, thus becoming osteocytes.[59] During the transition, osteoblasts undergo evident changes in their shape, accompanied by a significant reduction in the number and size of organelles.[60] It is assumed that an increase in the autophagic process occurs during this transition from osteoblasts to osteocytes. This increase is due to the need for faster organelle recycling and preservation of nutrients, while the cell develops its actin-rich prolongations, and adjusts to an environment more susceptible to hypoxia.[59] In fact, the expression of LC3 in osteocytes is greater than in osteoblasts.[61]

Osteocytes are long-lived cells located in gaps delimited by mineralized bone matrix and, consequently, are positioned in an environment more susceptible to hypoxia and to the accumulation of oxidative stress.[55] It is believed that under these conditions autophagy plays an important role in the survival of osteocytes.[59] Osteocyte-like cells have increased autophagic activity after undergoing nutrient deprivation and hypoxia in vitro, conditions that are similar to those found in osteocytes in vivo. In addition, in

response to calcium-mediated stress, a hypoxia-inducing transcription factor (HIF-1) positively regulates autophagy, indicating that low oxygen tension serves as a regulator of autophagy in this type of cell.[61] Low-dose glucocorticoid treatment has also been shown to induce autophagy in osteocytes in response to increased oxidative stress caused by treatment, preserving the viability of these cells.[62-64]

Studies indicate that autophagy also plays an important role in bone resorption by osteoclasts.[65] It has been reported that proteins involved in the autophagic pathway are important in the regulation of osteoclastogenesis,[66,67] indicating that this process participates both in bone formation and resorption. There is evidence that resorptive activity is decreased when bafilomycin, an autophagy inhibitor, is added to the culture of osteoclasts.[68,69] In mice, a mutation in the gene encoding the p62 protein has also been reported to result in a phenotype similar to Paget's bone disease, a condition in which there is excessive increase in bone remodeling and susceptibility to fractures.[70] In addition, it has been reported that an increase in autophagy occurs during osteoclastogenesis under conditions of hypoxia and increased oxidative stress caused by glucocorticoid treatment. In this case, it was suggested that autophagy would act as a protective factor reducing cell stress, increasing the formation and viability of osteoclasts.[71-73]

Deletions in genes encoding key proteins in the formation of the autophagosome (ATG5, ATG7, ATG4B and LC3) have been shown to cause changes in the brush border formation of osteoclasts, and, consequently, to reduce bone resorption and increase bone volume, thus preventing bone loss in mice after ovariectomy.[65] Some authors suggest that inhibition of autophagy in osteoclasts may serve as a possible therapeutic mechanism against bone diseases in which there is an excessive increase in bone resorption.[55,72] In fact, it has recently been observed in mice that pharmacological and genetic inhibition of autophagy reduces osteoclastogenesis and bone resorption, inhibiting bone loss caused by ovariectomy or glucocorticoid treatment.[74]

Recently, autophagy has also been pointed as an important factor in the process of bone growth. It was observed that the secretion of type II collagen by chondrocytes of the epiphyseal disc of mice is regulated by autophagy, under the influence of fibroblast growth factor 18 (FGF18).[28] However, the mechanisms by which autophagy regulates the secretion of type II collagen during bone growth are still not fully understood. A clinical trial of gene screening that included 618 adult Chinese subjects demonstrated that the expression of genes regulating the autophagic pathway may influence stature variation in this population.[75] Considering that autophagy increases the viability of chondrocytes, the authors report that this influence can be attributed to the protective role that autophagy exerts on these cells in the epiphyseal disc, thus influencing the growth of long bones.[76]

Other recent studies indicate that the activation of autophagy is also associated with the repair process of bone fractures.[77,78] In this case, it has been proposed that the increase in autophagy that occurs after a bone injury would act as a defense mechanism of the bone cells against cell stress, caused by the sudden reduction or interruption of the nutrient supply, due to bone fracture.[78] Thus, studies indicate that autophagy is a crucial factor for the maintenance of bone tissue homeostasis.

Autophagy, bone loss and osteoporosis: What is the evidence?

It has been widely reported that dysfunction in the autophagic pathway is involved in the development of various pathologies such as: neurodegenerative (e.g. Alzheimer's and Parkinson's)[79] and vascular[80] diseases, cancer,[81] diabetes,[82] obesity,[83] rheumatoid arthritis,[84] and osteoarthritis.[85] Studies suggest that dysregulation of autophagy may also be associated with the process of bone loss and subsequent osteoporosis.[27,86]

Recently, pharmacological inhibition with chloroquine and genetic inhibition by the selective deletion of the *Atg7* gene in monocytes have been shown to reduce osteoclastogenesis and decrease bone loss in animal models.[74,87] Administration of an antibody against sclerostin, an inhibitor of bone formation by osteoblasts, was able to prevent bone loss promoted by treatment with glucocorticoids in male mice.[88] In the study, treatment with high doses of glucocorticoids reduced the percentage of LC3-positive osteoblasts, an autophagic marker, thus reducing the viability of these cells and causing bone loss. Treatment with anti-sclerostin antibody, in turn, maintained the viability of osteoblasts by increasing autophagy in these cells and reducing bone loss caused by glucocorticoid treatment.[88] However, further studies are needed to understand the specific mechanisms by which sclerostin, or its antibody, regulates the autophagic pathway in bone cells.

Such findings indicate that autophagy contributes to the maintenance of bone mass, in part by maintaining the viability of osteoblasts. According to this idea, a deletion of the *FIP200* gene (essential for autophagosome formation) in osteoblasts has been reported to cause osteopenia in rats due to the decrease in bone formation by these cells.[89]

In osteocytes, the deletion of the *Atg7* gene (essential for autophagy initiation) has been shown to promote

bone mass decrease in 6-month-old male and female mice, similar to that caused by natural aging.[86] In another study, a relationship was observed between decreased autophagic activity in osteocytes and bone loss during aging in senile rat models.[29] Recently, this result has also been achieved in senile rat models in which rapamycin, an autophagy inducer, reduced osteoporosis by activating autophagy in osteocytes.[30]

These studies indicate that the mechanism by which autophagy reduces the bone loss caused by aging in these models seems to be related to the antioxidant effect of autophagy on bone cells. The fact that oxidative stress was higher in knockout animal models for *Atg7* reinforces this hypothesis.[86] Also, although estrogen deficiency is considered a major cause of bone loss and osteoporosis in postmenopausal women, studies have shown that increased oxidative stress in bone tissue is one of the major contributing factors to bone loss caused by aging.[59,90,91]

In short, the reduction of autophagy appears to promote increased oxidative stress, causing bone loss, whereas an increase in the autophagic pathway inhibits this effect.[29,30,86] According to this hypothesis, a significant inverse correlation was observed between the level of autophagy in osteocytes and the oxidative stress and bone loss caused by estrogen deficiency in the tibia of ovariectomized rats.[92]

Most evidence indicating a possible relationship between autophagic dysfunction and osteoporosis arises from studies in animal and in vitro models. In humans, however, there is still very little evidence to indicate such a relationship. A genetic screening study in humans revealed that, among the different pathways studied, the expression of autophagic pathway regulatory genes was the only one that showed a direct relationship with the variation in bone mineral density in the distal portion of the radius in 984 individuals.[93] The authors concluded that this relationship may indicate an important participation of the autophagic pathway in the development of osteoporosis.

The preclinical in vivo and in vitro studies included in our review indicate a relationship between the dysregulation of the autophagic pathway and osteoporosis. This possible relationship is summarized in Figure 2.

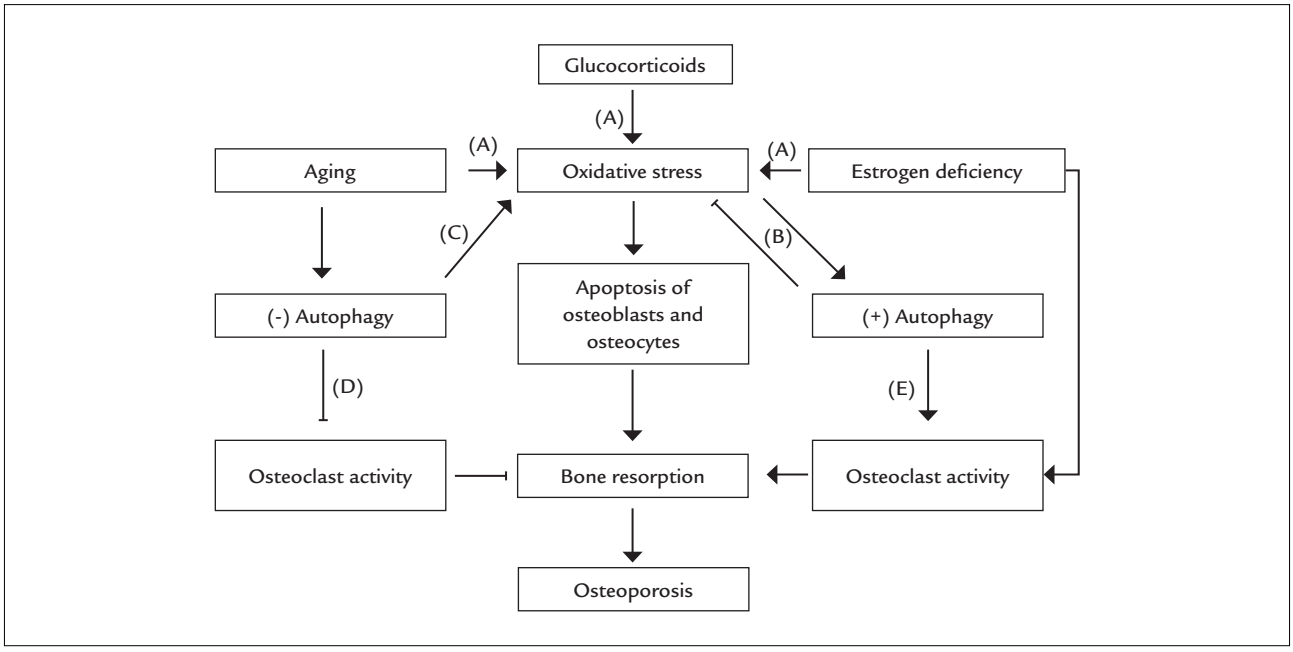

FIGURE 2 Diagram illustrating the possible relationships between autophagy and osteoporosis. (A) Aging, prolonged treatment with glucocorticoids and estrogen deficiency increase oxidative stress in bone tissue, inducing apoptosis of osteoblasts and osteocytes and increased bone resorption by osteoclasts, which induces bone loss and osteoporosis. (B) On the other hand, increased oxidative stress stimulates the autophagic (+) pathway, which reduces the deleterious effect of stress by degrading damaged proteins and organelles. Reduction of cell stress inhibits the apoptosis of osteoblasts and osteocytes, decreasing bone loss and osteoporosis. A reduction of autophagy (-) caused by aging results in increased oxidative stress (C), which can cause bone loss and subsequent osteoporosis. Autophagy is also essential for the viability of osteoclasts. Thus, while the reduction of autophagy decreases the formation and resorptive activity of these cells (D), the increase of autophagy stimulates these processes (E). Arrows indicate stimulatory action and bars indicate inhibitory action.

Conclusion

In conclusion, based on experimental in vivo and in vitro studies, there is a number of evidences that reinforce the existence of a correlation between the autophagy process and osteoporosis. Nevertheless, future studies, especially clinical trials, are needed to confirm the possible relationship between autophagic dysfunction and osteoporosis in humans, as well as to develop future therapies for the prevention and/or treatment of osteoporosis.

Resumo

Osteoporose e autofagia: qual é a relação?

A autofagia é uma via de sobrevivência celular pela qual proteínas e organelas não funcionais são degradadas nos lisossomos, para reciclagem e geração de energia. Assim, a autofagia é fundamental para a manutenção da homeostase e viabilidade da célula, agindo como um controle de qualidade que evita o acúmulo de estruturas desnecessárias e o estresse oxidativo. Um número crescente de estudos tem demonstrado que disfunções na via autofágica estão relacionadas ao surgimento de diversas doenças, como as neurodegenerativas e o câncer. Estudos também têm indicado que a autofagia exerce um importante papel para a manutenção da homeostase óssea; por exemplo, estudos *in vitro* e em animais e humanos mostram que disfunções da autofagia nas células ósseas estão associadas ao surgimento de doenças ósseas, como a osteoporose. Nesta revisão, foram abordados esses estudos, a fim de melhor esclarecer se há uma relação entre disfunção autofágica e osteoporose.

Palavras-chave: autofagia, tecido ósseo, osteoblasto, osteócito, osteoclasto, osteoporose.

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Induced pluripotent stem cells reprogramming: Epigenetics and applications in the regenerative medicine

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Article received: 5/23/2016
Accepted for publication: 5/30/2016

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http://dx.doi.org/10.1590/1806-9282.63.02.180

Summary

Induced pluripotent stem cells (iPSCs) are somatic cells reprogrammed into an embryonic-like pluripotent state by the expression of specific transcription factors. iPSC technology is expected to revolutionize regenerative medicine in the near future. Despite the fact that these cells have the capacity to self-renew, they present low efficiency of reprogramming. Recent studies have demonstrated that the previous somatic epigenetic signature is a limiting factor in iPSC performance. Indeed, the process of effective reprogramming involves a complete remodeling of the existing somatic epigenetic memory, followed by the establishment of a "new epigenetic signature" that complies with the new type of cell to be differentiated. Therefore, further investigations of epigenetic modifications associated with iPSC reprogramming are required in an attempt to improve their self-renew capacity and potency, as well as their application in regenerative medicine, with a new strategy to reduce the damage in degenerative diseases. Our review aimed to summarize the most recent findings on epigenetics and iPSC, focusing on DNA methylation, histone modifications and microRNAs, highlighting their potential in translating cell therapy into clinics.

Keywords: induced pluripotent stem cells, regenerative medicine, cell reprogramming, epigenetics, histones, microRNAs.

Introduction

Human embryonic stem cells have great potential for self-renewal and the ability to differentiate into all tissues of the body (except embryonic attachments),[1] forming an important source of material for regenerative medicine and cell therapy. However, the use of embryonic stem cells is limited by ethical and religious conflicts, as well as immunological incompatibility.[2] In order to reduce the damage caused by degenerative diseases, different strategies are being used in an attempt to optimize the use of embryonic stem cells.

The first strategy used was that of somatic cell nuclear content (SCNT) for unfertilized and enucleated oocytes.[3] However, the yield of this technique is still very low and the cells obtained can present phenotypic and gene expression abnormalities.[4] Another strategy is the fusion of somatic cells with embryonic cells, reprogramming their genome.[5] However, although fusion-induced reprogramming is very efficient (about 95%), the resulting hybrid cells lack therapeutic potential due to their tetraploidy, as well as the presence of exogenous genes from the pluripotent cells used in the fusion.[5] Therefore, a search for new strategies for the efficient use of cells with an embryonic profile is still needed.

In 2006 Takahashi and Yamanaka managed to induce pluripotency in mouse fibroblasts (MEF) from the expression of four embryonic transcription factors (Oct4, Sox2, Klf4, c-Myc), currently known as OSKM or "Yamanaka factors." These cells were called induced pluripotent stem cells (iPSCs).[6] The iPSC are similar to embryonic cells in terms of morphology, gene expression, differentiation status and epigenetic pattern, both in culture, as well as in vivo.[7] This mechanism of reprogramming somatic cells into embryonic stem cells resulted in the Nobel Prize in

medicine for Yamanaka in 2012, shared with John b. Gurdon.[8] Since then, several surveys have been developed to explore this technology. In fact, several researchers were able to reprogram somatic cells (postmitotic) into iPSC using the aforementioned strategy.[9-11]

The advantage of using this method is that it allows the derivation of pluripotent cells from the donor, reducing the risk of rejection by the immune system. In addition, this method provides a platform to study the molecular mechanisms of genetic and chronic diseases, minimizing the ethical, religious and political conflicts and opening up new perspectives for regenerative medicine. However, although cell identity can be modified by the ectopic expression of transcription factors, the efficiency of reprogramming remains low (0.1 to 3%) and its cost is high.[11,12]

The low reprogramming efficiency of iPSC is associated with residual epigenetic memory of the tissue from which they were derived, which complicates the reprogramming process. Recent studies show that despite iPSC sharing common characteristics of pluripotency and self-renewal capacity, these cells still retain an epigenetic memory.[13-15] In addition, there is evidence that the reprogramming process involves complete remodeling of the existing somatic epigenetic memory, followed by the establishment of a new "epigenetic signature" that conforms to the type of cell to be differentiated.[16] Therefore, the epigenetic memory becomes a barrier in the process of cellular reprogramming. This fact highlights the need for new studies investigating the epigenetic changes associated with cellular reprogramming in an attempt to improve the efficiency and effectiveness of the iPSC created, as well as their clinical application. As such, our review aimed to gather information about the epigenetic factors (DNA methylation, changes to histones and microRNAs) associated with iPSC reprogramming efficiency. In addition, we have brought together the clinical studies using iPSC as cell therapy.

Epigenetics and iPSC

Waddington was the first researcher to use the term epigenetics in 1942 to explain how the genome interacts with the environment during the development process.[17] Therefore, any reversible and inheritable change in the functional genome that does not alter the sequence of DNA nucleotides refers to epigenetics.[18] Several pathologies are associated with epigenetic changes.[19-22]

The reprogramming efficiency of iPSC is also directly related to epigenetic changes such as DNA methylation and the epigenetic memory of the source cells.[23] The iPSC reprogramming process can be divided into three distinct phases, called pre-iPSC, intermediate and full reprogramming. The reprogramming process is extremely slow, with low efficiency (0.1 to 3%) and high cost,[11,12] and depends on suitable levels of gene expression in each phase and specific epigenetic changes.[24] Djuric and Ellis compared the epigenetic changes that occur during the reprogramming process with a "seven headed dragon," where a series of changes is necessary for efficient reprogramming, namely: 1) Endogenous reactivation of genes related to cell pluripotency, Nanog and Oct4; 2) Chromatin changes, such as trimethylation in H3K27 and changes in H3K4; 3) Hypomethylation of heterochromatin; 4) Reactivation of the inactive X chromosome; 5) Maintenance of DNA methylation marks; 6) Silencing the retrovirus that induces pluripotency; and, finally, 7) Two- or three-dimensional chromatin changes and location of nuclear subdomains.[24] Therefore, the control of epigenetic factors during reprogramming may improve the induction of iPSC and their efficiency.[25]

Epigenetic changes in iPSC reprogramming

DNA methylation in iPSC reprogramming

DNA methylation is an epigenetic mechanism involved in many important cellular processes such as cell proliferation and differentiation, transcriptional repression, genomic imprinting, organization of chromatin and inactivation of the X chromosome.[26] Thus, changes in the DNA methylation profile are associated with the appearance of many degenerative diseases.

Studies show that DNA methylation is considered a crucial epigenetic barrier in the reprogramming of iPSC.[27-31] For the expression of genes essential for reprogramming, such as Oct3/4 and Nanog, demethylation of cytosines is necessary in the respective promoter regions.[32] Thus, inhibition of methylation by enzymes or interfering RNA may be an option to improve this process.[33] Mikkelsen et al. showed that the use of 5-azacytidine (AZA) inhibits the enzyme DNA methyltransferase 1, assisting in DNA demethylation, which may favor the reprogramming of iPSC.[33] Another study noted an improvement in reprogramming efficiency using DNA hydroxylase (Tet1), an enzyme that is able to oxidize 5-methylcytosine and, after subsequent replications, promotes DNA demethylation by reactivating Oct4 gene transcription, favoring the reprogramming process.[31] In addition to the use of AZA and the enzyme Tet1, other researchers have used ascorbic acid (vitamin C). Vitamin C acts as a cofactor, stimulating hypomethylation and consequently increasing the activity of histone H3K36 demethylase. This process results in an improvement of cellular reprogramming efficiency.[34-36]

The aforementioned studies demonstrate the importance of DNA demethylation in cell reprogramming. Several studies have attempted to improve these and other epigenetic mechanisms in order to improve both the quality and efficiency of iPSC reprogramming. Another relevant topic for such improvement is the change in histones during the reprogramming process.

Changes to histones in iPSC reprogramming

Histones are basic proteins rich in lysine and may suffer several epigenetic changes. Most of these modifications happen in the N-terminal region of the histone, with the exception of ubiquitination, which occurs in the C-terminal region of H2A and H2B.[37] Epigenetic modifications to histones may either promote or inhibit gene transcription by changing the level of chromatin folding.[38,39]

Taking into consideration the epigenetic changes in histones in iPSC, H3 is the histone researched the most, as it is directly related to genes expressed during embryonic development, such as Oct3/4, Sox2 and Nanog. It has already been demonstrated that methylation of H3K27 is associated with the suppression of various genes, and that persistent trimethylation of the lysine 27 of histone 3 (H3K27me3) blocks reprogramming by repressing the chromatin region associated with the target genes of the stem cells. However, the methylation of H3K4 is associated with the activation of different embryonic genes.[24] In an attempt to improve the performance of iPSC and reach the ideal conditions for the induction of pluripotency, by reducing the "epigenetic memory" in somatic cells, different strategies that directly or indirectly affect the methylation/acetylation of H3 have been used.[12] Several researchers have demonstrated that it is possible to perform the induction of pluripotency without the use of Yamanaka factors, using only chemical compounds/molecules that interfere with the enzymes that control the chromatin structure.[40]

Recently, Rais et al. showed that the inhibition of Mbd3 – a subunit of the NuRD complex responsible for the deacetylation of histones, remodeling of nucleosomes and gene expression inhibition – is able to reactivate the genes expressed during embryonic development and improve the efficiency of iPSC reprogramming by almost 90%, both in human as well as mouse cells.[41]

Many other strategies have been tested in order to improve the iPSC reprogramming process, such as the use of small molecules like Forskolin (FSK),[42] BIX-01294,[43] valproic acid (VPA – HDAC histone deacetylase inhibitor)[44] and vitamin C.[45] Therefore, the induction of pluripotency of iPSC can only occur with the use of small molecules.[46-48] The authors advocate the use of such because they are not immunogenic, with greater yield and easy production. Thus, the use of chromatin modulators can increase efficiency in the iPSC reprogramming process.[49] To do so, it is necessary to use a small molecule that is able to demethylate the DNA in the promoter region and change specific regions in histones.

MicroRNAs in iPSC reprogramming

MicroRNA or miRNA are important tools for regulating gene expression in post-transcriptional iPSC by promoting pluripotency to modulate the stability of messenger RNA (mRNA) and suppress the signs of differentiation during the self-renewal of embryonic stem cells.

MiRNAs also modulate the signaling cascades that are necessary for maintaining the pluripotent state.[50] Wang et al. noted that the loss of function in the enzyme Dicer and DGCR8, proteins essential for the biogenesis of microRNAs in the embryonic stem cells of mice, present two different phenotypes: 1) reduction in proliferation due to cell cycle arrest in G1; and 2) resistance to differentiation, which reveals a close relationship between microRNAs, differentiation and the pluripotency of cells.[51,52]

MicroRNAs are small non-coding RNA molecules. They have 18-25 nucleotides (nt), and are derived from a larger precursor. The processing of microRNAs occurs as follows:

1. After DNA transcription by RNA polymerase II or III the primary microRNA is formed (pri-miRNA). This may be presented in the shape of a fork. The pri-miRNA first undergoes processing by the enzyme ribonuclease (RNase) nuclear III-like DROSHA. The specificity of the cleavage in this step is guided by the DGCR8 protein, which acts as a "molecular ruler," positioning the DROSHA at a distance of 11 nucleotides from the base of the pri-miRNA loop. After cleavage, a pre-miRNA (precursor miRNA) is released, formed by about 60-70 nt.[53]
2. The pre-miRNA is actively transported to the cytoplasm by exportin-5 (Exp5), when this protein is linked to its Ran-dependent GTP cofactor. In the cytoplasm, it undergoes another cleavage process, where it loses the loop and is reduced to a miRNA duplex approximately 18-25pb in length. This last stage of processing is conducted by DICER, an RNAse-III type enzyme, aided by the TRBP protein.[53]
3. Finally, the RNA-binding protein TRBP recruits a multimeric complex denominated RISC (RNA-induced silence complex), which includes the protein Argonaute 2 (in mammals) as the main component.

Only one of the microRNA duplex strands remains in the RISC complex (guide strand), with the other being degraded (passenger strand). The RISC complex is able to identify and bind to target messengers RNAs in region 3'UTR through complementarity of bases in the "SEED" region of the miRNA (nucleotides 2-8 from the 5' end) in order to inhibit its translation or promote its adenylation and degradation.[53]

MicroRNAs that promote the reprogramming of iPSC

- miR 290-295 (cluster): These constitute more than 70% of the entire population of microRNAs in the embryonic stem cells of mice. miR 291-3 p, miR-294 and miR-295 are part of this cluster and indirectly promote the transition of genes associated with entry into the G1-S phase, blocking *Cdkna* (p21), a suppressor of the Cyclin E/Cdk2 complex, and regulator of the cell cycle. After its transfection into MEFs there is an increase of 0.01-0.05% to 0.1-0.3% in cell reprogramming efficiency.[54]
- miR 302-367 and miR 371-373 (cluster): These miRNAs suppress the expression of MBD2 (methyl-CPG-binding domain protein 2), which works like a demethylase in cells, resulting in increased expression of Nanog and conversion of completely reprogrammed iPSC. They are also able to reduce expression of the inhibitors in the G1-S phase, as well as increasing the kinetics of the mesenchymal-epithelial transition (MET) required for reprogramming.[55,56] Only the use of miR-302a/b/c/d and miR-367 is able to reprogram adult cells, and with greater efficiency, when compared to the Yamanaka method.[57] Data demonstrate that miR-302 in conjunction with Yamanaka factors inhibits NR2F2 (nuclear receptor subfamily 2, group F, member 2) and improves reprogramming efficiency through indirect positive regulation of Oct4.[58]
- miR-200b and -200c, miR-205: These promote MET via signaling of transforming growth factor β (TGF-β) and, in conjunction with the Yamanaka factors, exclude the need for signaling of bone morphogenetic protein (BMP) during the initial reprogramming phase.[59]
- miR-93, miR-106: They suppress the expression of TGF-β and p21, leading to MET and increased proliferation.[60]
- miR-135b: This is highly expressed during the reprogramming process, regulating the expression of TGF-β, IGFBP5 (insulin-like growth factor binding protein 5) and Wisp1 (inducible-signaling pathway protein 1), which are involved in the expression of extracellular matrix genes.[61]

MicroRNAs that are barriers to iPSC reprogramming

- miR Let-7 (cluster): This inhibits the Pou5f1/Oct4, Sox2, Nanog and Tcf3 targets – pluripotency factors – stabilizing a differentiated state. In addition, this miRNA inhibits the translation of CDK4, repressing the transition of the G1-S-phase. MiR Let-7 forms a negative feedback circuit, providing a molecular mechanism that facilitates the decision between self-renewal and differentiation of cells.[50,62]
- miR-34a, miR-34b/c: miR-34a represses the expression of Nanog, Sox2 and c-Myc. Taken in conjunction, miR-34a and miR-34b/c target p53 (tumor suppressor gene), holding an essential role in the containment of somatic reprogramming.[63]

These studies show that miRNAs can be important tools in the mediation of iPSC reprogramming without the need for the ectopic expression of pluripotency induction factors, including OSKM factors. The tables below present a summary of different approaches and their effects on iPSC reprogramming. Table 1 is related to changes in DNA methylation, Table 2 is related to modifications in histones and Table 3 is related to the use of miRNAs. In addition, Figure 1 presents these changes in summarized form.

Clinical application of iPSCs

The main discussion about the use of iPSC in regenerative medicine is related to their ability to transform into cancer cells. Incomplete reprogramming of iPSC may be associated with the emergence of various mutations.

In addition to the impact on iPSC reprogramming, OSKM factors are associated with the development of tumors. Oct4 is highly expressed in the cervical cancer cell line. Deletion of Sox2 is associated with regression of melanomas. The Klf4 and c-Myc factors regulate genes involved in cell growth and proliferation.[64] However, over the last 10 years following the discovery of iPSC, this technology has undergone several changes, such as the use of episomal plasmids that are not integrated into the genome, diminishing the carcinogenic potential of these cells.[65] Currently, several methods are used to develop iPSC lines, including the use of plasmids, transposons, adenovirus, Sendai virus, miRNA and chemical compounds, minimizing mutagenic factors. Given these advances, iPSC have been used in pre-clinical tests and clinical trials.

The first transplant of iPSC in humans occurred in Japan in the second half of 2014, in a 70-year-old patient with age-related macular degeneration (AMD). This pa-

TABLE 1 Epigenetic DNA changes.

Authors	Year	Species	Type of cell	Technology	Epigenetic changes	Results
Mikkelsen et al.[33]	2008	Mice	Fibroblasts	5-azacytidine (AZA)	Inhibitor of DNA methyltransferase	Promotes the demethylation of pluripotency genes
Mikkelsen et al.[33]	2008	Mice	Fibroblasts	shRNA/siRNA	Reduced expression of DNMT1 using siRNA/shRNA	Demethylation and complete reprogramming of cells
Popp et al.[28]	2010	Mice	Embryonic	AID	DNA demethylation	Clears all standard demethylation of the genome
Doege et al.[29]	2012	Mice	Fibroblasts	Tet2	Generation of 5-hydroxymethylcytosine (5hmC) through the oxidation of 5mC	Hydroxylation of pluripotency sites (Nanog and Esrrb) favoring a greater number of iPSCs
Costa et al.[30]	2013	Mice	Embryonic	Tet1/Tet2	Physical interaction with Nanog	Hydroxylation of pluripotency sites (Oct4 and Esrrb)
Gao et al.[31]	2013	Mice	Fibroblasts	Tet1	DNA demethylation and activation of Oct4	Better induction of pluripotency

iPSCs: induced pluripotent stem cells.

TABLE 2 Epigenetic changes in histones.

Authors	Year	Species	Type of cell	Technology	Epigenetic changes	Results
Huangfu et al.[44]	2008	Mice	Fibroblasts	VPA/TSA/SAHA	Inhibitor of histone deacetylation	Prevents histone deacetylation, increasing reprogramming efficiency by more than 100X compared to OSKM
Shi et al.[43]	2008	Mice	Fibroblasts	BIX-01294	G9a methyltransferase inhibitor	Reduces histone H3K9 methylation, facilitating transcription. Improves reprogramming efficiency compared to Oct4 and Klf4
Esteban et al.[35]	2010	Humans	Fibroblasts	Vitamin C	Nanog promoter, Oct4/ Histone demethylation	Decreases senescence during reprogramming
Liang et al.[46]	2010	Mice	Fibroblasts	Sodium butyrate	Histone deacetylase (HDAC) inhibitor	Facilitates reprogramming mediated by c-Myc
Liang et al.[48]	2012	Mice	Fibroblasts	Lentivirus increasing the expression of Kdm2b	Activation of genes in the initial phase of reprogramming	Decreased H3K36me2 levels, contributing to reprogramming
Onder et al.[47]	2012	Mice	Fibroblasts	epz004777	DOT1L inhibitor (Histone methyltransferase specific for H3K79)	With the reduction of DOT1L, a reduction in the methylation of H3K79 occurs, improving the reprogramming by increasing transcription factors such as Nanog and Lin28
Chen et al.[45]	2013	Mice	Fibroblasts	Vitamin C	Increases Kdm3 and Kdm4 – Lysine-specific demethylase	Reduces H3K9me2 and H3K9me3, important early in the reprogramming of iPSCs
Hou et al.[42]	2013	Mice	Fibroblasts	3-deazaneplanocin A (Dznep)	Histone methyltransferase inhibitor	Better reprogramming occurs with inhibition of histone methyltransferase by preventing methylation in the arginine and lysine residues of histones

OSKM: Oct4, Sox2, Klf4 and c-Myc; VPA/TSA/SAHA: valproic acid/trichostatin A/suberoylanilide hydroxamic acid; iPSCs: induced pluripotent stem cells.

TABLE 3 Epigenetic changes using microRNAs.

Authors	Year	miRNA	Species	Type of cell	Mechanism of action	Results
Judson et al.[54]	2009	290-295	Mice	Fibroblast	Blocks p21, leading to increased cell cycle proteins	Promotes the G1-S phase transition (proliferation), indirectly activating pluripotency factors
Li and He[50]	2012	Let-7	Mice	Embryonic stem cell	Reduced CyclinD/Cdk4 regulation	Blocks G1-S phase
Subramanyam et al.; Lin et al.[55,56]	2011	302-367	Mice	Fibroblast	Inhibits TGFII-β	Promotes MET
Choi et al.[63]	2011	34a and 34b/c	Mice	Fibroblast	Reduced regulation of p21 and p53	Indirectly blocks pluripotency factors
Li et al.[61]	2014	135b	Mice	Fibroblast	Reduced expression of TGF-β, Igfbp5, and Wisp1	Promotes MET
Zare et al.[49]	2015	124-128	Humans	Fibroblast	Regulate the development of neurons	Promotes the migration, maturation and differentiation of neurons, maintaining adequate gene expression and repressing unwanted genes

MET: mesenchymal-epithelial transition.

thology affects around 700,000 people in Japan, and is the most common form of blindness in people aged over 60, causing progressive loss of the retinal pigment epithelial monolayer. The transplant lasted around two hours and, according to the researchers, the patient did not suffer adverse effects, and there were an improvement in the morphology of the macula and neovascularization.[66]

This clinical trial was carried out by the group of Professor Takahashi, co-author of the manuscripts that won Professor Yamanaka the Nobel Prize in Medicine in 2012. However, in March 2015, clinical testing intended to treat six patients was suspended due to regulatory changes in regenerative medicine in Japan.[67]

Other clinical trials are currently underway to test the effectiveness of cellular therapy with iPSC in the treatment of AMD, Parkinson's disease, spinal cord injury, diabetes and myocardial infarction.[68] Preliminary results have not yet been presented.

Maintenance of the epigenetic factors of the iPSC after reprogramming has also been used to understand the molecular pathways involved in the development of diseases, development of new drugs and personalized medicine.[69] The first study conducted of this kind used a model of neuropathic disease. The authors reprogrammed fibroblasts from patients with Riley-Day syndrome and monitored in vitro splicing of the IKBKAP (mutation associated with the disorder). Furthermore, the researchers also evaluated candidate drugs for reversal of the splicing. The study of iPSC gains relevance in this case, due to the inability of accessing the tissues affected by Riley-Day syndrome.[70] Other studies have been developed along this line,[69] and are promising in the context of drug development. Figure 2 summarizes the use of iPSC.

Conclusion

Despite the improvement recently seen in iPSC reprogramming by up to 100 times, when compared to Yamanaka factors,[44] the yield remains relatively low and high costs. Another problem is the long period for full iPSC reprogramming and the high cells proliferation rates associated with a greater chance of developing cancer.[71] Takahashi and Yamanaka suggest considering using iPSC with allogeneic transplantation in regenerative medicine, which would improve the effectiveness of the treatment of certain diseases such as spinal cord injury, which requires quick treatment without waiting for the time taken for reprogramming.[11] Furthermore, there is a need for better understanding of how the reprogramming interventions influence the epigenetic memory of the reprogrammed cells. Despite advances in iPSC reprogramming, certain questions have emerged: 1) Is it possible to completely erase the somatic epigenetic memory by associating the different treatments mentioned?; 2) Could "forced" reprogramming cause long-term damage, such as the development of cancer or other diseases?; 3) Is it possible to replace embryonic stem cells with iPSC in regenerative medicine? In spite of the extraordinary progress achieved recently in the use of iPSC, the deepening of ongoing studies and realization of new studies are necessary in order to elucidate

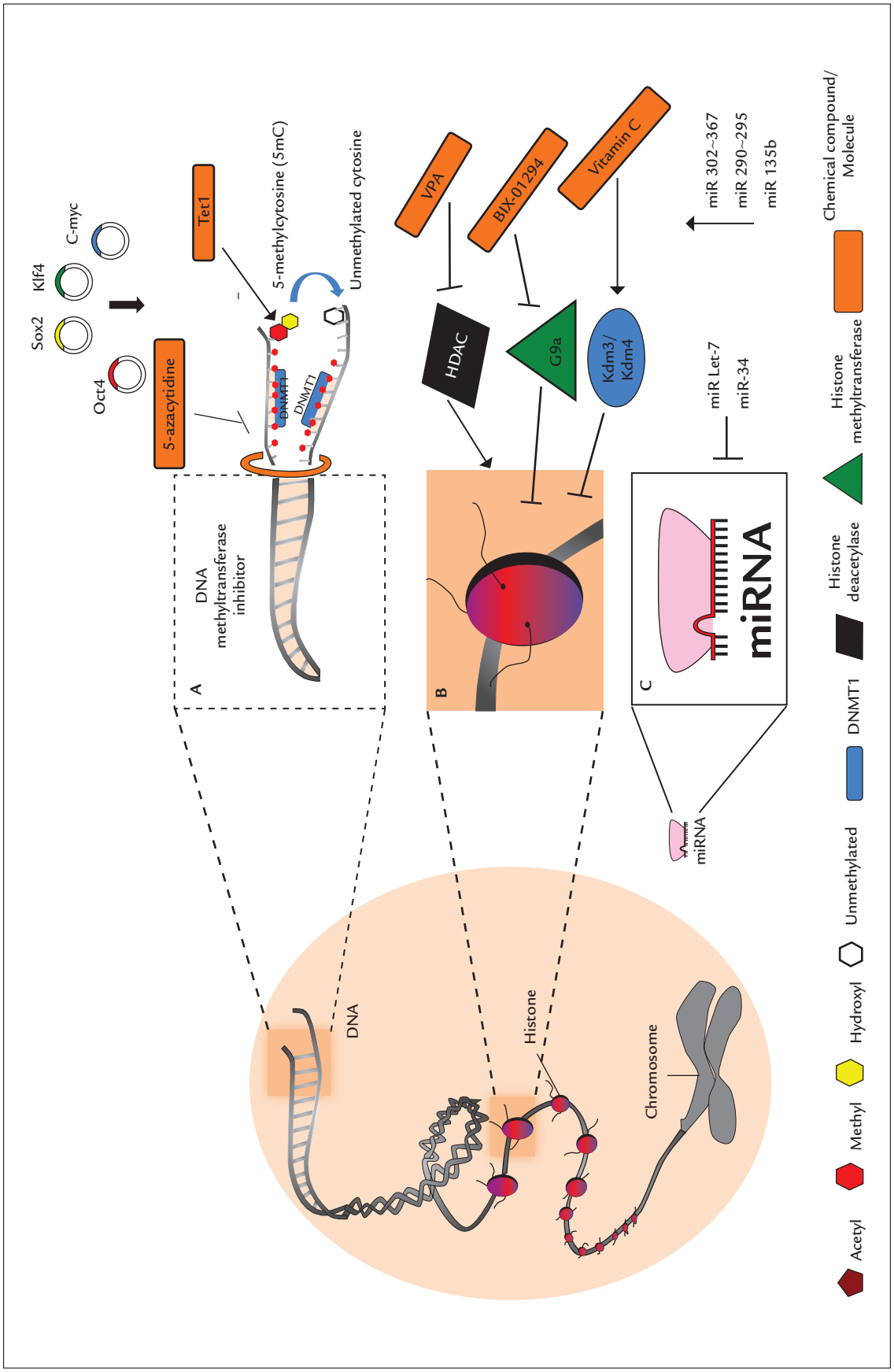

FIGURE 1 Epigenetic factors and iPSC reprogramming efficiency. The ectopic expression of Yamanaka factors, Oct4, Sox2, Klf4 and c-Myc (OSKM) are able to lead to DNA demethylation and reprogramming of somatic cells. A. The use of Tet1 (DNA hydroxylase) and of 5-azacytidine (inhibitor of the enzyme DNA methyltransferase) improves the reprogramming efficiency of iPSC cells. B. The use of valproic acid (VPA), BIX-01294 and vitamin C favors reprogramming through inhibition of histone deacetylase (HDAC), histone methyltransferase (G9a) and activation of the demethylases Kdm3/Kdm4, respectively. C. miRNAs 302-367, 290-295, 135b are able to increase reprogramming efficiency by promoting the progression of the cell cycle. miR Let-7 inhibits the cell cycle, and miR-34 inhibits the translation of p53, decreasing reprogramming efficiency.

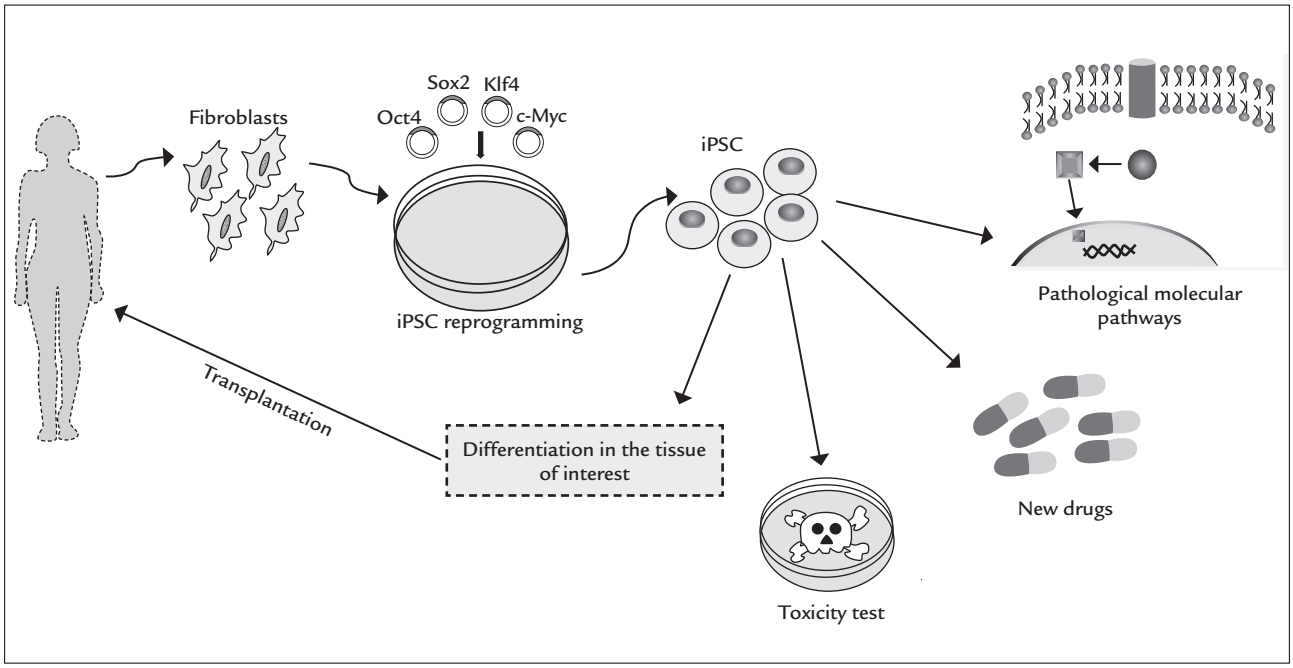

FIGURE 2 Somatic cells are reprogrammed into induced pluripotent stem cells (iPSCs). These cells are differentiated in the tissue of interest and transplanted in an attempt to reduce the damage caused by degenerative diseases. In addition, iPSCs are also being used in pre-clinical and clinical tests.

the mechanisms of the aforementioned changes, optimizing their application in regenerative medicine.

Resumo

Células-tronco de pluripotência induzida: papel da epigenética na reprogramação e sua aplicabilidade clínica

As células-tronco de pluripotência induzida (CTPI) ou do inglês *induced pluripotent stem cells* (iPSCs) são células somáticas reprogramadas para o estado embrionário por meio da expressão de fatores ectópicos de transcrição específicos, tornando-as um alvo promissor para a medicina regenerativa. Apesar das CTPI compartilharem características embrionárias, como pluripotência e capacidade de autorrenovação, elas possuem uma baixa eficiência de reprogramação, sendo a memória epigenética uma das principais barreiras nesse processo. A epigenética é caracterizada por alterações reversíveis e herdáveis no genoma funcional que não alteram a sequência de nucleotídeos do DNA. Dentre as diferentes modificações epigenéticas, destacam-se metilação de DNA, alterações em histonas e microRNA. Atualmente, sabe-se que o processo de reprogramação efetivo das CTPI envolve um completo remodelamento da memória epigenética somática existente, seguido pelo estabelecimento de uma "assinatura epigenética" que esteja de acordo com o novo tipo de célula a ser diferenciada. Modificações epigenéticas personalizadas são capazes de melhorar o rendimento e a efetividade das CTPI geradas, abrindo uma nova perspectiva para a terapia celular. Nesta revisão reunimos as principais informações sobre os fatores epigenéticos que afetam a reprogramação das CTPI, bem como seus benefícios na aplicação da terapia celular.

Palavras-chave: células-tronco de pluripotência induzida, medicina regenerativa, reprogramação celular, epigenética, histonas, microRNA.

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Bariatric surgery in individuals with liver cirrhosis: A narrative review

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Summary

Introduction: Bariatric surgery has become the gold standard treatment for morbid obesity, but there is no consensus regarding its safety and efficacy among individuals with chronic liver diseases.
Objective: To critically evaluate the existing evidence on literature about bariatric surgery in individuals with liver cirrhosis.
Method: Narrative review performed by means of an online search in the MEDLINE and LILACS databases.
Results: Bariatric surgery is safe and effective in individuals with chronic liver disease without clinical decompensation or significant portal hypertension. Individuals with severe liver function impairment present significantly higher surgical morbidity and mortality. Among candidates to liver transplantation, surgery may be performed before, after and even during transplantation, and there is a predominant trend to perform it after. Vertical sleeve gastrectomy seems to be the most adequate technique in this group of subjects.
Conclusion: Bariatric surgery is safe and effective in individuals with compensated cirrhosis without significant portal hypertension, but presents higher morbidity. Among candidates to liver transplantation and/or individuals with severe portal hypertension, morbidity and mortality are significantly higher.

Keywords: liver diseases, obesity, bariatric surgery, liver cirrhosis, liver transplantation.

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Article received: 6/23/2016
Accepted for publication: 6/26/2016

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http://dx.doi.org/10.1590/1806-9282.63.02.190

Introduction

Since the second half of the 20th century, the world has seen a significant increase in the prevalence of overweight and obesity. According to the World Health Organization (WHO), there are more than 1.4 billion adults over their ideal weight, including more than 200 million men and almost 300 million women that are obese. Currently, 65% of the world's population lives in countries where obesity and overweight are responsible for more deaths than malnutrition.[1]

Likewise, the prevalence of obesity among cirrhotic individuals who are candidates for liver transplantation has almost doubled since the 1990s, reaching more than 30%.[2,3] Furthermore, obesity and metabolic syndrome have presented an increasingly significant causal relationship with chronic liver disease.[4] Non-alcoholic fatty liver disease (NAFLD) is currently the third leading cause of liver transplantation in the United States of America (USA). Also, there is evidence that, if suitable diagnostic criteria are applied, about 2/3 of cases characterized as cryptogenic cirrhosis are actually caused by NAFLD.[5,6] It is predicted that in 2030, NAFLD will be the leading cause of liver transplantation in the USA.[7]

In recent years, bariatric surgery has become the gold standard treatment for morbid obesity, leading to better results compared to clinical treatment.[8] However, among obese patients with chronic liver disease, there is no consensus regarding the most effective and safest therapeutic

strategy. The association between obesity and cirrhosis is a complex situation for various reasons. Encouraging the adoption of lifestyle changes in individuals with severe liver diseases is difficult, and bariatric surgeries present greater risks, with less favorable rates of morbidity and mortality. In addition to the isolated risk of liver disease, in patients with NAFLD and obesity, the existence of other comorbidities, such as atherosclerotic cardiovascular disease, diabetes, hypertension, dyslipidemia, metabolic syndrome and chronic nephropathy, is common.[9] Given that the intersection between obesity and NAFLD is increasingly common, a deeper understanding of these interconnections and the possibilities of more suitable and safer management for the proper treatment of both conditions is necessary.

Objective

To conduct a critical analysis of the existing literature on the realization of bariatric surgery on patients with liver cirrhosis.

Method

A narrative review of the literature was conducted via an online search of the MEDLINE (via Pubmed) and LILACS (via Bireme) databases, using as keywords "bariatric surgery," "liver diseases" and "liver cirrhosis." The articles were located and reviewed, with an emphasis on those reporting on the results of bariatric surgical techniques in individuals with cirrhosis and/or chronic liver diseases.

Results and Discussion

In subjects with mild to moderate liver disease without cirrhosis, several studies have demonstrated the occurrence of regression of NAFLD after bariatric surgery, including individuals with significant fibrosis.[10,11] This improvement occurs not only due to weight loss, but is also related to complex mechanisms linked to the structural and biochemical changes caused by the surgery, such as improved insulin sensitivity, increased incretin and adipokine activity, reduction of chronic inflammation and decreased lipid supply in the portal system.[12]

The main risk factors for postoperative impairment after performing bariatric surgery in chronic liver diseases are portal hypertension and hepatocytic insufficiency. In relation to severe cirrhotic liver transplant candidates, the choice of the most appropriate surgical technique and the time for completion of the surgery are relevant aspects that have not been completely established, especially due to the scarce literature on these topics.

In patients with severe cirrhosis, perioperative morbidity and mortality are higher than those observed in the obese population. Takata et al.[13] assessed 15 patients with severe liver disease (six of which were cirrhotic patients) treated with vertical sleeve gastrectomy and noted a 33% loss of excess weight after one year, with perioperative complications in two (13.3%) patients, both of whom were cirrhotic. In 26 transplant candidates submitted to vertical sleeve gastrectomy assessed by Lin et al.,[14] perioperative complications were noted in 23.1%, with no mortality. The average loss of excess weight after one year was 50% and seven patients were submitted to transplant, without complications related to the bariatric surgery.

In a retrospective study based on a database analysis at the national level in the USA, Mosko et al.[15] noted higher mortalities in clinically compensated (0.9%) and non-compensated cirrhotic patients (16.3%) when compared to individuals free of liver disease (0.3%). Furthermore, in centers with a low volume of bariatric surgery, mortality reached 41% among individuals with decompensated cirrhosis.

Incidental diagnosis of liver cirrhosis in the intraoperative period of bariatric surgery is not rare, and is reported in 1 to 4% of cases.[16,17] Dallal et al.[18] analyzed a sample in which 90% of patients with cirrhosis had been diagnosed incidentally in the intraoperative period of the bariatric intervention and noted that among individuals with compensated cirrhosis (Child-Pugh A), the Roux-en-Y gastric bypass had a mortality rate similar to the general population, but with more episodes of transient renal dysfunction, greater surgery time and more bleeding and the need for blood products. Woodford et al.[19] studied 14 patients with intraoperative diagnosis of cirrhosis during placement of an adjustable gastric band, without changes in hepatocyte function or portal hypertension, and did not note significant mortality or morbidity. Pestana et al.[20] conducted a retrospective study comparing patients with Child-Pugh A cirrhosis and without portal hypertension, noting similar morbidity and mortality and considering surgery as being well-tolerated and safe therapeutic option in patients with compensated liver diseases and mild portal hypertension.

In a systematic review, Lazzati et al.[21] found a 66% loss of excess weight within two years, comparable to that found in the general population. Vertical sleeve gastrectomy was the procedure conducted the most, and perioperative mortality was similar to that in the general population. However, the morbidity rate, in particular the frequency of reoperations, and the mortality rate in the first year were higher. The heterogeneity of the studies and the small number of individuals analyzed, even after the compilation of data, have been identified as

factors that generate potential biases and limit the findings. Another systematic review, conducted by Jan et al.,[22] showed favorable results comparable to those of the general obese population in relation to weight loss and the resolution of comorbidities. On the other hand, the authors underscore that the risks are significantly higher, with 21.3% surgical morbidity, 1.6% perioperative mortality and 2.4% late-onset mortality. The risk of decompensation of liver function was also high (6.5%) and should be taken into consideration. The main results of the studies evaluated are presented in Table 1.

Among severe cirrhotic patients that are candidate to liver transplant, the choice of technique to be employed is fundamental due to two key issues: potential damage to the absorption of immunosuppressive medication and the possibility of endoscopic access to the biliary tree. There are no studies examining the pharmacokinetics of immunosuppressants in liver transplant patients undergoing bariatric surgery. In kidney transplant patients undergoing gastric bypass, however, there are reports of a need for larger doses of tacrolimus, sirolimus, mycophenolate sodium and cyclosporine.[23] With regard to access to the biliary tree, stenoses are common after deceased donor transplants, occurring in up to 17% of cases.[24] Due to these factors, vertical sleeve gastrectomy appears to be the most appropriate technique in this group of patients, as it does not cause significant malabsorption and enables endoscopic access to the biliary tree.

The ideal time for bariatric surgery in liver transplant candidates is another controversial issue. There is a possibility of performing the procedure before, after and even during transplantation. A relevant concern in this regard is the impact of obesity on the outcome of the transplant. Recent studies have reported that operative mortality, two-year survival and graft viability are similar in obese and non-obese individuals. Perioperative morbidity is slightly higher.[2,3] Performing bariatric surgery in non-compensated transplant candidates leads to higher morbidity and mortality, including the occurrence of anastomotic fistulas, which often reaches 12.5%.[21,22]

Recently, there has been growing interest in the possibility of endoluminal treatments for obesity in individuals with high surgical risk. An endoscopic intragastric balloon implant in this group of patients, which is an attractive alternative, has presented satisfactory results in studies conducted by Choudhary et al.[25]

A case report by Campsen et al.[26] showed the realization of adjustable gastric band implantation during a liver transplant had satisfactory results after 6 months. Heimbach et al.[27] reported the combined realization of liver transplantation and vertical sleeve gastrectomy in seven patients with one case of a fistula on the staple line and zero mortality. The combined option is interesting because it reduces the number of surgical approaches in high-risk patients. On the other hand, this approach requires complex logistics (especially the concomitant availability of transplant and bariatric teams) and can combine serious complications that are not related to either procedure.[21,22,26,27] Therefore, in patients with non-compensated cirrhosis or with moderate to severe portal hyper-

TABLE 1 Main results of bariatric surgery in individuals with liver diseases.

Study	N	Type of study	Surgical technique	Morbidity	Perioperative mortality	Late mortality
Takata et al.[13]	15	Retrospective cohort	Vertical sleeve gastrectomy and gastric bypass	13.3%	0	0
Lin et al.[14]	26	Retrospective cohort	Vertical sleeve gastrectomy	23.1%	0	0
Mosko et al.[15] (Compensated cirrhosis)	3,888	Retrospective cohort	Several	NR	0.9%	NR
Mosko et al.[15] (Decompensated cirrhosis)	62	Retrospective cohort	Several	NR	16.3%	NR
Dallal et al.[18]	30	Retrospective cohort	Gastric bypass	30%	0	3.3%
Woodford et al.[19]	14	Prospectively collected database analysis	Adjustable gastric band	14.3%	0	0
Shimizu et al.[31]	23	Prospective database analysis	Vertical sleeve gastrectomy and gastric bypass	34.8%	0	4.3%
Lazzati et al.[21]	56	Meta-analysis	Several	23.2%	0	5.3%
Jan et al.[22]	122	Meta-analysis	Several	21.3%	1.6%	NR

N: number of patients; NR: not reported.

tension, conducting the liver transplantation first and bariatric surgery *a posteriori* is preferred. The clearest advantage of this option is the selection of patients who have had a favorable outcome after transplantation and developed or maintained obesity, now with improvements in hepatocyte function and portal hypertension. Morbidity is higher than in the general population (reoperation rate of up to 33%) but mortality is similar.[21,22,28-30]

Conclusion

In individuals with liver disease and preserved hepatocytic function and without significant portal hypertension, bariatric surgery is an effective and safe therapeutic option, with results close to those of patients without liver disease. In these cases, the techniques used the most are vertical sleeve gastrectomy and Roux-en-Y gastric bypass. In patients with severe liver diseases, candidates for transplantation or post-transplant patients, the most appropriate technique is vertical sleeve gastrectomy. Performing surgery prior to transplantation is significantly worse than in the general population and should be avoided. Considering the current encouraging results of liver transplantation in obese patients, the post-transplant approach seems to be the most appropriate. The realization of prospective controlled studies with large samples is required so that we can obtain more definitive conclusions.

Conflict of interest

The authors declare no conflict of interest.

Resumo

Cirurgia bariátrica em indivíduos com cirrose hepática: uma revisão narrativa

Introdução: A cirurgia bariátrica tornou-se nos últimos anos o tratamento padrão-ouro para a obesidade mórbida; porém, entre obesos portadores de hepatopatia crônica, não existe consenso a respeito de sua segurança e efetividade.
Objetivo: Análise crítica da literatura existente sobre a realização de cirurgia bariátrica em portadores de cirrose hepática.
Método: Revisão narrativa por meio de pesquisa *online* nas bases de dados Medline e Lilacs.
Resultados: As cirurgias bariátricas em indivíduos cirróticos sem descompensação clínica levam a resultados satisfatórios. Já indivíduos com hepatopatia grave apresentam morbidade perioperatória e mortalidade significativamente maiores do que as observadas na população obesa sem hepatopatia. Em candidatos a transplante hepático, a cirurgia pode ser realizada antes, durante ou após o transplante, havendo uma tendência predominante à realização após o transplante. A gastrectomia vertical parece ser a técnica mais adequada nesse grupo de pacientes.
Conclusão: A cirurgia bariátrica é segura e efetiva em portadores de cirrose hepática compensada e sem hipertensão portal; porém, apresenta maior morbidade. Em candidatos a transplante e/ou indivíduos com hipertensão portal significativa, a morbimortalidade é significativamente maior.

Palavras-chave: hepatopatias, obesidade, cirurgia bariátrica, cirrose hepática, transplante de fígado.

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