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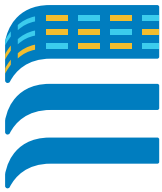
Brazilian Journal of Health and Biomedical Sciences

VOL. 22, Nº 2, JUL-DEZ/2023



BJHBS

Brazilian Journal of Health and Biomedical Sciences

Vol. 22, número 2, julho-dezembro/2023

Rio de Janeiro

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Partially supported by

FAPERJ
Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro

Classified in

QUALIS

CATALOG AT SOURCE
UERJ/REDE SIRIUS/CBA

Brazilian Journal of Health and Biomedical Sciences. – V. 22, n. 2 (jul.-dez.2023) . – Rio de Janeiro: HUPE, 2002-
v. : il. (some color.)

Semestral 2002-.
Available at: bjhbs.hupe.uerj.br
Previous title: Revista Hospital Universitário Pedro Ernesto.

1. Ciências médicas – Periódicos. 2. Saúde – Periódicos. I. Hospital Universitário Pedro Ernesto.

CDU 61

Librarian: Thais Ferreira Vieira - CRB - 5302

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Summary

Obesity: Individual problem or social problem?

Obesity has reached epidemic proportions globally, with millions of people dying each year due to excess weight or obesity. Although the health sciences community has seemingly always been concerned with the health of the population and worked hard to identify conditions that affect human health and alleviate suffering, the contemporary pathologization of obesity, which has historically even been considered a sign of health and social power (Figure 1), began in the late 1950s following studies carried out by life insurance companies. Since then, many studies have linked excess weight to an increased risk of diabetes, cancer, and cardiovascular disease, which led the World Health Organization to declare a global epidemic of obesity. Obviously, the fight against obesity has been included in health promotion strategies. Even during the conception of collective health strategies, the fight against obesity has always been understood as the responsibility of the individual for his or her well-being. However, the time when obesity was considered as simply an individual health problem seems to be gone. New and dangerous discourses about the obese emphasize that they not only harm themselves but the community as a whole, because the direct and indirect health costs related to obesity are shared by everyone. In addition, obesity has been associated with losses in productivity and other discourses that could potentially fuel prejudice towards overweight people. What should be done? Getting fat is incredibly easy!

For many years, the food industry has encouraged the consumption of processed and ultra-processed food in large quantities and in family-sized packaging, under the premise that this arrangement is more economical. Currently, following the introduction of new medications for the treatment of diabetes and obesity, the impact on consumption is so great that the ingestion of family-sized foods is decreasing, and certain food companies are losing money for the first time. In other words, we are moving from a beginning marked by the pathologization of obesity due to a need for profit by life insurance companies, through the strengthening of scientific evidence from the health sciences about the negative consequences of obesity, to the reinvention of food companies that are suffering the impact of modern treatments against obesity. It is incredible how it seems that the wheel always revolves around money and the discourse that the collective is more important than the individual. Even when these situations seem to occur just for temporary convenience.

Figure 1. Brazil. 6,400 Reis, 1810/1 R. 14,34 g Gold (.917) Laureate bust of Prince Regent John facing right, legend around, with date and mintmark R (Rio de Janeiro 1694-date) below. Overdate 1810/1. Reverse: Crowned Portuguese arms topped by royal crown. KM# 236.1

Eloísio Alexsandro da Silva Ruellas
Editor In Chief

DOI: 10.12957/bjhbs.2023.80912

BJHBS

Insulin-like growth factor-1 short-period therapy improves non-alcoholic fatty liver disease in obese mice

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Abstract

This study seeks to evaluate Insulin-like Growth Factor 1 (IGF-1) short-period therapy in Non-Alcoholic Fatty Liver Disease (NAFLD) as it relates to western diet-induced obesity. For this purpose, 21-day-old male Swiss mice were divided into a control group (CG, N=8), which was fed a standard diet, and an obese group (GO, N=16), which was fed a western diet, rich in saturated fat and simple carbohydrates, for 12 weeks. In the 11th week, part of the animals in the obese group (N=8) received a daily subcutaneous injection of recombinant human IGF-1 (100µg/kg/day) during seven consecutive days (GO+IGF-1). Biometric and metabolic parameters, intraperitoneal glucose tolerance test (IGTT), quantitative analysis of liver steatosis, quantitative analysis of collagen in liver and expression of immunoperoxidase of alpha-smooth muscle actin (a-SMA) were analyzed. Our data demonstrated that IGF-1 short-term treatment was able to improve obesity-related biometric and metabolic parameters. In addition, it promoted the recovery of liver parenchyma, thereby reducing steatosis and fibrosis, thus demonstrating an important hepatoprotective action.

Keywords: Non-Alcoholic Fatty Liver Disease; Liver Fibrosis; IGF-1; Obesity.

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BJHBS, Rio de Janeiro, 2023;22(2):81-90
DOI: 10.12957/bjhbs.2023.79959
Received on 22/03/2023. Approved on 03/09/2023.

Introduction

Despite its multifactorial nature, obesity is deeply related to nutritional habits, including the consumption of foods with high calorie content, rich in saturated fat, salt and sugar, usually called the western diet.[1,2] In addition to an increase in fat deposits, obesity is linked to comorbidities, including type 2 diabetes mellitus (T2DM), nonalcoholic fatty liver disease (NAFLD), hypertension, hyperlipidemia, chronic kidney disease, and cardiovascular disease, leading to increased mortality in obese individuals.[3]

Non-alcoholic fatty liver disease (NAFLD) is one of the most prevalent chronic liver conditions and an important risk factor for liver cirrhosis and hepatocellular carcinoma.[4] NAFLD is the spectrum of liver disease in which hepatic steatosis, the macrovesicle accumulation of triglyceride in hepatocytes,[5,6] is considered the ultimate effector of lipotoxic liver injury.[7]

Insulin-like growth factor-1 (IGF-1) is an anabolic growth hormone associated with proliferation, growth and cellular metabolism, and low IGF-1 plasma levels have been correlated with obesity[8,9] and NAFLD.[10] Therefore, this study sought to investigate the therapeutic potential of short-period of recombinant IGF-1 treatment on NAFLD in an experimental model of obesity induced by the western diet.

Methods

Animals and experimental design

Animals were cared for in accordance with the guidelines of the Ethics Commission on Animal Use of the Biology Institute of the State University of Rio de Janeiro (CEUA/026/2017), established under standard international protocols.

Male Swiss mice at the 21st day after birth were housed under standard conditions of temperature and controlled humidity with a 12h light/dark cycle. Animals were randomly divided into a control group (CG, N=8), which was fed a standard AIN93G diet (65.6% carbohydrates, 17.3% proteins, and 17.1% lipids) and an obese group (OG=16), which was fed a Western diet rich in saturated fat and simple carbohydrate from clarified butter (ghee) (43.3% carbohydrates, 14% proteins, and 42.7% lipids) (PragSoluções, Brazil).

Diets were administered during 12 weeks with free access to water and food. After 11 weeks, half of the obese group mice (N=8) received a daily subcutaneous injection of 50µl of human recombinant IGF-1 (100µg.kg−1.day−1) (PeproTech) in saline solution for seven consecutive days (OG+IGF-1). The other groups received injections of 50µl of phosphate buffer saline.

Biometric and metabolic parameters

Body weight and naso-anal length were assessed after 12 weeks, and the Lee index was calculated by the formula: cube root of body weight (g)/naso-anal length (cm)×1,000. Epididymal and retroperitoneal fat were excised and weighed. In addition, the liver steatosis index was analyzed by liver mass (g)/body mass (g). Fasting glucose was measured with a glucometer (Accu-Chek Active, Roche Diagnostics, Germany) on the euthanasia day (111th day of life) and fasting insulin was evaluated by radioimmunoassay (Insulin IRMA KIT; ref. IM3210, Beckman Coulter, Miami). The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index was calculated by using the formula: [fasting insulin (µUI/mL)×fasting glucose (mmol/L)]/22.5.

Intraperitoneal glucose tolerance test (IGTT)

Intraperitoneal glucose tolerance test was performed after 6 hours of fasting after 12 weeks of diet. An intraperitoneal injection of glucose (1g/kg of body weight) was administered, and blood droplets were collected from the tail vein just prior to glucose administration (time 0) and after 30, 60, 90 and 120 minutes. The blood glucose level was measured using a glucometer and AccuChek Active test strips (Roche Diagnostics, Germany).

Quantitative analysis of liver steatosis

The liver was fixed in 4% formaldehyde, and paraffin sections were stained with hematoxylin and eosin. To quantify liver fat droplets, 15 random fields per animal, collected from non-serial

sections, were captured in a light microscope with CCD camera. The analysis was conducted with STEPanizer software. Results were expressed as density/area (µm2).

Quantitative analysis of collagen in liver

The liver was collected, fixed in 4% paraformaldehyde, dehydrated in increasing series of alcohol, clarified in xylol, and included in paraffin. Sections of 5.0µm were obtained and stained with Picro-Sirius Red (0.1% solution of Direct Red 80, Sigma-Aldrich), which stains collagen fibers in red, and hematoxylin. To quantify hepatic collagen deposition, 15 random fields per animal, collected from non-serial sections, were captured with a 40x objective in a light microscope with CCD camera. The analysis was made with Image Pro Plus 3.0 software by densitometry of areas stained in red. The results were expressed as a percentage of stained area over the total field.

Immunoperoxidase of alpha-smooth muscle actin (a-SMA)

The liver was fixed in 4% formaldehyde and included in paraffin. Sections were then incubated with monoclonal mouse anti-rat alpha-smooth muscle actin primary antibody (Santa Cruz Biotechnology), followed by anti-mouse biotinylated secondary antibody, streptavidin-peroxidase and, finally, DAB chromogen. The sections were then stained with hematoxylin and mounted with Entellan. To quantify a-SMA expression in the experimental groups, 15 random fields per animal were captured in a light microscope with a CCD camera and then analyzed with Image Pro Plus 3.0 software.

Statistical analysis

Data were expressed as mean±standard error of the mean and statistical significance was assessed by one-way or two-way analysis of variance with Holm-Sidak post-test, with $P \leqslant 0.05$ being considered statistically significant.

Results

Biometric and metabolic parameters

From week 5 of western diet administration, the OG demonstrated a significant increase in body mass compared to the CG ($P<0.0001$), which persisted until the end of the experiment (Figure 1A). However, the OG + IGF-1 showed 15% of body mass reduction compared to the OG group, reaching values similar to the CG (Table 1).

After IGF-1 treatment, the values of Lee index, epididymal fat, and retroperitoneal fat mass similar to the CG were also restored in the OG+IGF-1, with no difference in naso-anal length between groups. In addition, the OG+IGF-1 showed a 13% of reduction in the steatosis hepatic index when compared to the OG group (Table 1).

IGF-1 treatment improved fasting glycemia compared to the OG, although it did not fall to the levels seen in the CG. Moreover, only one week of treatment was not enough to improve insulin levels and HOMA-IR (Table 1).

IGTT analysis demonstrated that the OG showed an almost two-fold increase in glucose intolerance compared to the CG. After IGF-1 treatment, the OG+GF-1 showed an improvement in the glucose tolerance curve (−22%) compared to the OG (Figure 1C-D).

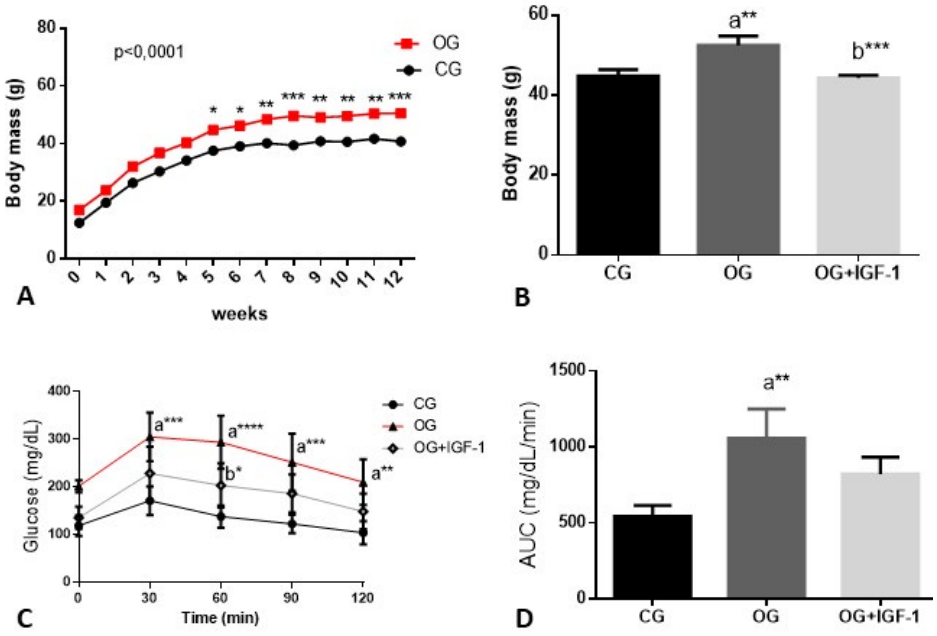

Figure 1. Body mass evolution, Intraperitoneal glucose tolerance test and Area under the curve

Legend: (A) Body mass evolution of the control group (CG) and the obese group (OG) for 12 weeks. P value of change in time using repeated measures model effect. (B) Body mass on the day of euthanasia of CG, OG, and OG + IGF-1. (C) Intraperitoneal glucose tolerance test. P value of change in time using repeated-measures model effect. (D) Area under the curve. Data are expressed as mean ± SEM. "a" significant difference compared to CG, "b" significant difference compared to OG. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

Source: The authors (2022).

Table 1. Biometric and metabolic parameters

	CG	OG	OG+IGF-1
Body mass (g)	44,7±1,6	52,4± 2,3 [a]	44,3±0,6 [b]
Naso-anal length (cm)	8,9±0,2	9,4±0,1	9,3±0,1
Lee Index	346,5±4,48	419,5±3,53 [a]	318,6±13,53 [b]
Fasting blood glucose (mg/dL)	102,8±9,4	175,0±5,3 [a]	139,8±9,4 [a,b]
Epididymal fat (g)	0,82±0,1	2,24±0,3 [a]	1,32±,0,1 [b]
Retroperitoneal fat (g)	0,30±0,06	0,91±0,12 [a]	0,52±0,06 [b]
Insulin levels (mUI/mL)	50±8,4	124,3±5,7 [a]	159±19,2 [a]
HOMA-IR	2,18±0,3	4,71±0,6 [a]	4,96±0,62 [a]
Liver Steatosis (g/g)	0,04110±0,00094	0,05131±0,00283 [a]	0,04451±0,00124 [b]

Legend: Data are expressed as mean ± SEM; n = 8/group. a: Significant difference compared to CG. b Significant difference compared to OG. *P < 0.04;**P<0.0019; ***P < 0.0005; ****P < 0.0001

Source: The authors (2023).

Steatosis quantification

The light microscopy analysis of liver sections stained with hematoxylin and eosin (Figure 2) showed that hepatic parenchyma of the OG has an intense degree of steatosis. Both macrovesicular (large vacuoles within each hepatocyte, which make the nucleus eccentric) and microvesicular (despite numerous vacuoles within each cell, the nucleus remains in its central position) steatosis was observed. However, the predominance of macrovesicular steatosis is evident. The OG+IGF-1 showed a significant improvement of the hepatic parenchyma, with an almost total reduction of macrovesicular steatosis; however, some hepatocytes with microvesicular steatosis remained. The quantification of fat droplets demonstrated that the OG (1097±135.5) presented a significant increase in fat droplets per area in relation to the CG (321.9±80.75). IGF-1 treatment was able to reduce hepatic steatosis by 43.4% in the GO+IGF-1 (621.3±53.32) compared to the GO (Figure 2D).

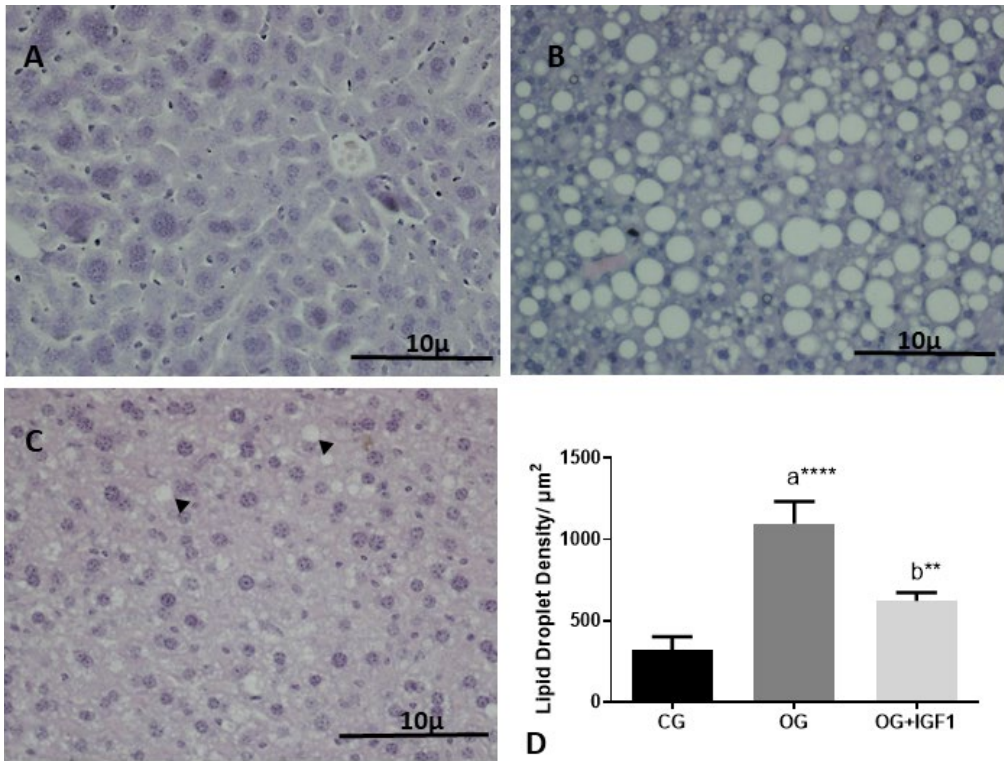

Figure 2. Histological liver sections stained with Hematoxylin and Eosin from (A) CG (control group), (B) OG (obese group) and (C) OG + IGF-1. (D) Quantitative analyses of Lipid Droplet Density/ µm².

Legend: Data are expressed as mean ± SEM. Arrowhead indicate hepatocytes with microvesicular steatosis. "a" significant difference compared to CG, "b" significant difference compared to OG. ** P < 0.0015 ****P < 0.0001.

Source: The authors (2023).

Liver fibrosis quantification

Picro Sirius Red staining demonstrated that collagen deposition in the liver was higher in the OG (Figure 3B) than the CG (Figure 3A). Treatment with IGF-1 reduced the fibrosis area by 43% compared to the OG (Figure 3D).

For fibrosis analysis, liver sections were stained with Picro-Sirius Red, which shows collagen deposition allowing the quantification of these areas. Compared to the CG (Figure 3A), the OG

showed greater deposition of collagen in the hepatic parenchyma, mainly around the vessels and between the hepatocyte cords (perisinusoidal area) (Figure 3B). In contrast, the OG+IGF-1 demonstrated a decrease in collagen deposition, which was mainly restricted to the area around the vessels, like the CG.

Quantitative analysis of Picro-Sirius Red stained area corroborated microscopic analysis that showed a significant increase of 30% in collagen deposition in the OG compared to the GC, characterizing the adverse remodeling of the extracellular matrix. However, after IGF-1 treatment, a significant reduction of 43% occurred in the fibrosis area compared to GO (Figure 3D).

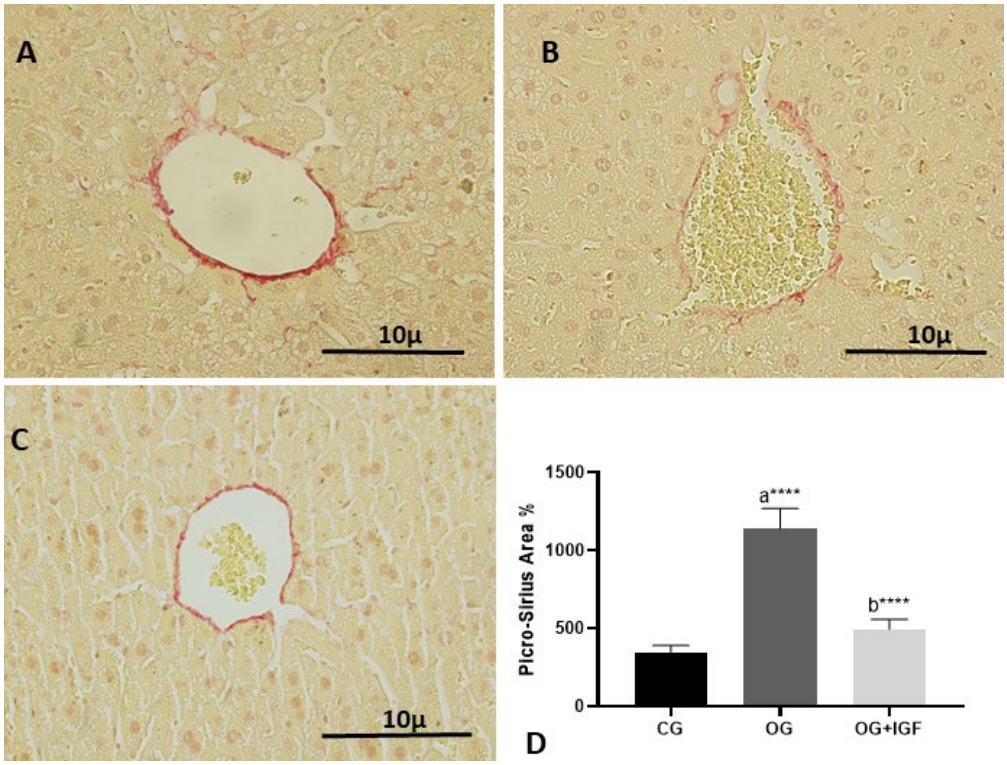

Figure 3. Histological heart sections stained with PicroSirius Red from (A) CG (control group), (B) OG (obese group) and (C) OG + IGF-1. (D) Quantitative analyses of PicroSirius stained area (D)

Legend: Data are expressed as mean ± SEM. "a" significant difference compared to CG, "b" significant difference compared to OG. ****P < 0.0001

Source: The authors (2023).

Immunoperoxidase of α-SMA

Analysis of α-SMA expression in the liver demonstrated that this biomarker of activated myofibroblast has an extremely low expression in the CG (Figure 4A). However, the OG showed increased α-SMA expression between hepatocyte cords and around the centrilobular vein (Figure 4B). After a short period of IGF-1treatment, hepatic parenchyma of OG+IGF-1 demonstrated an evident decrease in the α-SMA expression (Figure 4C).

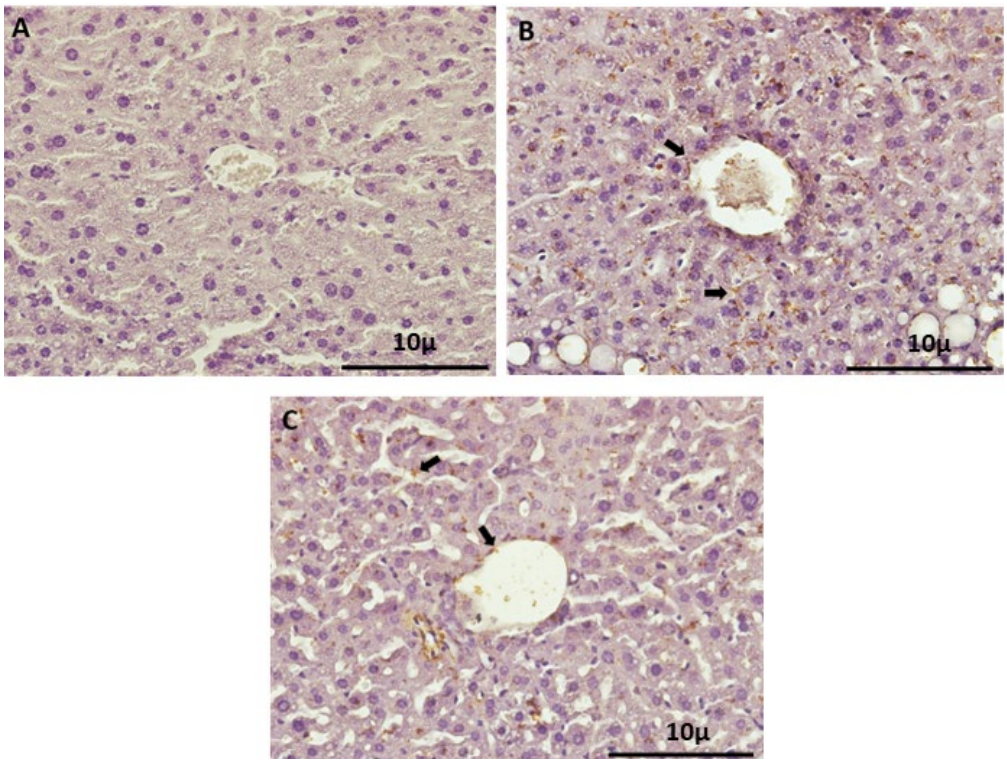

Figure 4. Histological heart sections immunostained with the smooth muscle alpha-actin-specific antibody, showing its staining in brown (arrows) and nuclei counterstained with hematoxylin, in purple.

Legend: (A) CG (control group); (B) OG (obese group) (C) OG + IGF-1.

Source: The authors (2023).

Discussion

The nutritional profile of the global population has been changing in recent decades with an increasing consumption of ultra-processed foods, with a high content of simple carbohydrates and saturated fat (western diet). Consequently, we observe an increase in caloric consumption at the expense of its expenditure, which results in alarming rates of obesity. In this study, we reproduced this dietary pattern, using the model described by Tikellis and colleagues[2] in male Swiss mice, already used by our group for some years,[11,12] in order to evaluate the therapeutic potential of IGF-1 in NAFLD induced by western diet on the restructuring of the liver parenchyma.

The present study corroborated the obesogenic character of the western diet showed by our previous studies, which found increases in body mass, adiposity and glycemia.[11,12] IGF-1-treated mice even showed a significant reduction in body mass after only one week of treatment, presenting the same body mass as the CG. IGF-1 activates the (PI3K)/Akt pathway, leading to anabolic effects and increasing lean mass through protein synthesis.[11,13] It also promotes an increase in lipid oxidation and inhibition of lipogenesis, preventing generation of new adipocytes,[14] which explains the reduction in adiposity in the treated group.

Obesity resulting from the excessive increase of calorie consumption in the western diet promotes the deposit of fat in the liver, leading to hepatic steatosis. Consequently, fatty acids accumulate in hepatocytes, resulting in a state of lipid cell hypertrophy. The excessive concentration of intracellular lipid in the hepatocyte causes steatosis, giving the hepatic parenchyma two distinct histological characteristics. In the first, microvesicular, the cytoplasm of the he-

patocyte is affected by small lipid vacuoles and the nucleus is located in the center of the cell. On the other hand, the macrovesicular steatosis is characterized by large vacuoles filled with lipids throughout the cytoplasm with consequent restriction of the nucleus to the periphery of the cell.[15]

For steatosis evaluation, we initially estimated hepatic steatosis, measured by the correction of liver weight (g) by the body mass (g), which showed a significant increase in the OG ratio when compared with the CG, indicating enlargement of the liver caused by fat accumulation. However, the OG+IGF-1 displayed a decrease in liver mass. To corroborate these initial results, the liver parenchyma was analyzed by light microscopy. We observed a significant amount of lipid droplets throughout the hepatic parenchyma of the OG, which is characteristic of hepatic steatoses, both microvesicle and macrovesicle. However, animals treated with IGF-1 for only one week showed a decrease in the density of lipid droplets, which were reduced to some regions of microvesicle hepatic steatoses, which demonstrated an improvement in the liver parenchyma of the mice. Nishizawa and colleagues[16] in a mouse model with GH deficiencies (SDR), revealed that these mice presented hepatic steatoses, hepatic fibrosis and increased oxidative stress according to NAFLD phenotype. In this model, however, treatment for four weeks with either GH or IGF-1 was sufficient to reverse the phenotype, with IGF-1 infusion having a particularly beneficial effect on fibrosis compared with GH infusion.

Liver steatoses promote a lipotoxic environment characterized by oxidative stress, lipid peroxidation, mitochondrial dysfunction and extensive hepatocellular death.[17] Kupffer cells lead to an inflammatory phenotype and secretion of inflammatory cytokines, such as IL-6, TNF-α and IL-1β. In addition to Kupffer cells, other immune cells are recruited into the tissue, contributing to the inflammatory profile.[18] In this context, sinusoidal cells, as well as resident and recruited immune cells, secrete pro-fibrotic factors, such as TGF-β and reactive oxygen species (ROS).[19] Such events stimulate fibrogenesis, since they activate the hepatic stellate cells, responsible for the deposition of extracellular matrix, altering the liver parenchyma and liver functionality.[20,21]

Hepatic stellate cells immune-activated by cell infiltration and liver injury undergo a phenotypic transdifferentiation, and start to express actin smooth muscle (α-SMA).[22] and to produce an extracellular matrix component, such as I, III and IV.[18,23] The accumulation of collagen is accompanied by an imbalance between the consequences of tissue matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). In murine fibrotic hybrids, the increase in MMPs was accompanied by an increase in TIMP-1.[24] This creates a direction for fibrogenesis in the MMP/TIMP balance with a shift in MEC synthesis and therefore fibrogenesis.[25]

Our results corroborated findings that the western diet promotes liver fibrosis in NAFLD patients and showed an increased fibrotic area in the OG[26]. After a single week of treatment, we observed reduced fibrosis in the hepatic parenchyma. In vitro experimental model reports an IL-6 decreasing in HepG2 cells, indicating the anti-inflammatory effect of IGF-1,[27] also demonstrated in mice CCl4 model, such as reducing oxidative stress and liver fibrosis,[28] through activation of the AKT pathway, which our group observed in previous work on the heart.[11]

Our data showed greater collagen deposition in the areas close to the portal triad, around the central-lobular vein and in the perisinusoidal spaces, which coincided with the area marked by α-SMA, an activated HSC marker (myofibroblast) in the OG. These results indicate that the western diet triggered a lipotoxic inflammation responsible for the activation of the fi-

brogenic profile of hepatic stellate cells. The OG+IGF-1, after a short-term treatment, showed a significant reduction of hepatic fibrosis area, indicating the hepatoprotective action of IGF-1.

A NAFLD study model, using db/db mice fed a methionine-choline-deficient diet treated with IGF-1, showed a reduction in pro-fibrotic markers, such as procollagen 1a1 and collagen 4a1, and inflammatory markers, such as Il-1β and Il – 6, by PCR. In addition the number of α-SMA positive cells was reduced, indicating a direct effect of IGF-1 on activated hepatic stellate cells that would promote their senescence, assessed by β-galactosidase activity both in vivo and in vitro. IGF-1 also promoted the increase of Mmp9 and a reduction of Timp1, thereby helping to resolve the fibrosis.[29]

Another study, which used transgenic mouse models (SMP8-IGF-I) to induce a cirrhotic state with carbon tetrachloride (CCl4), showed that SMP8-IGF-I mice exhibited decreased α-SMA expression and morphological improvement of the hepatic parenchyma, which restricts the activation of hepatic stellate cells and decreases fibrogenesis.[30] These data confirm our results, in which treatment with IGF-1 is related to a lower expression of α-SMA in the hepatocyte cords, which promotes the improvement of the liver.

Conclusion

Short-term treatment with IGF-1 was effective for the recovery of hepatic parenchyma in obese mice, which contributes to the improvement of NAFLD and represents a promising therapeutic approach.

Financial support

This work was supported by the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Rio de Janeiro (FAPERJ) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Conflict of interest

The authors declare that they have no conflict of interest in this study.

Ethical standards

All experimental procedures were approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro (CEUA 002/2017) and comply with the principles contained in Brazilian Law no. 11.794/2008.

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Influence of hamstring flexibility on the knee joint position sense

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Abstract

Introduction: Different factors have been put forward as positive or negative influences on the knee joint position sense; however, the effects of hamstring flexibility have not been the object of extensive research. Objective: To study the influence of hamstring flexibility on the knee joint position sense. Methods: The knee joint position sense of 31 adults was tested actively to extension to a 45° range of knee flexion, in both the dominant and non-dominant limb. Hamstring flexibility was assessed through the sit and reach test. Based on the results, participants were divided into high and low flexibility categories. Intergroup analysis and tests of the association between flexibility and repositioning errors were performed. Results: No significant differences were found between the two categories of flexibility and repositioning accuracy. Similarly, no significant associations were found between flexibility and repositioning errors ($p>0.05$). Conclusions: These results suggest that hamstring flexibility does not affect knee repositioning accuracy, implying that both lower and higher flexibility do not impair the knee joint position sense.

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BJHBS, Rio de Janeiro, 2023;22(2):91-97
DOI: 10.12957/bjhbs.2023.79502
Received on 28/12/2022. Approved on 20/07/2023.

Keywords: Hamstring; Flexibility; Joint Position Sense; Knee.

Introduction

Joint Position Sense (JPS) is a submodality of proprioception that measures the ability of an individual to memorize a given position and to actively or passively reproduce it, without the aid of vision.[1] Knee proprioception is mainly ensured by joint (Pacinian corpuscles and Golgi and Ruffini endings) and muscle mechanoreceptors (Golgi tendon organs and muscle spindles).[2,3] The respective contributions of these sources of afferent information have been debated, and research has established that the greatest contribution to JPS comes from the muscle mechanoreceptors,[4,5] especially the muscle spindles.

Muscle spindles are responsible for providing information about muscle length when being stretched, in order to consciously understand limb position.[6] Several authors state that these receptors can provide afferent information over the entire physiological range of motion of the joint.[7,8]

Different factors have been proposed as positive or negative influences on the knee JPS.[9-11] Earlier research on the acute effects of flexibility training incorporating muscle stretching exercises proved its beneficial effect on enhancing joint range of motion, but also its deleterious effect on some aspects of athletic performance, such as strength and power.[12-14] However, the effect of hamstring flexibility on knee repositioning accuracy has not been the subject of thorough investigation. To the best of our knowledge, only one study has assessed the association between flexibility and proprioceptive acuity,[15] which concluded that hamstring flexibility negatively affects knee JPS. However, the results of the study reveal a negative correlation between flexibility values and repositioning errors, which contradicts the conclusions of the study, since lower repositioning errors signify improved, not worse, accuracy. Therefore, further research is needed to clarify this question.

In this sense, the aim of this study was to investigate the influence of hamstring flexibility on the knee JPS, and, more specifically, its influence on knee extension repositioning accuracy.

Material and methods

Study design and sample

The study was approved by Ethics Committee of the Fernando Pessoa University. Each participant signed an informed consent that complies with the Helsinki Declaration of the World Medical Association, ensuring data anonymity and confidentiality, and barring use for any other purpose except this research. Participants were also informed that they could cease their participation in the study at any time without repercussions or need for justification.

Thirty-one adults (12 males; 19 females) participated in this research, with a median (interquartile range) age of 21.00 (1.00) years and a median (interquartile range) Body Mass Index (BMI) of 23.73 (5.39) kg/m.^{2}

All participants were male or female students (aged between 18 and 30) recruited from the university community, with no history of injuries to the lower limbs in the previous 6 months. Excluded from participation were all those with a history of knee surgery; cardiorespiratory, neurological, vestibular or oncological pathology; taking medication that might affect motor control (analgesics, NSAIDs, myorelaxants, antibiotics); and participants who were pregnant or breast-feeding at the time of the study. Determination of the dominant limb was conducted according to the guidelines established by Porac and Coren.[16]

Assessment of Knee Joint Position Sense

The Knee Joint Position Sense (KJPS) was assessed using a 2D video system and placing markers over 4 bony protuberances (lateral malleolus; head of the fibula; lateral epicondyle of the femur; and halfway between the great trochanter and the lateral epicondyle of the femur),[17] held in place with double-sided adhesive tape. Joint angles were later calculated using Kinovea software.

A goniometer was used to set the target angle of 45º of knee flexion[17] in order to assess repositioning accuracy to extension (in a seated position). In both tests, after the target angle was set passively, participants were instructed to actively hold the position for 5 seconds, then to return to the starting position (90º flexion), and immediately afterwards to actively reposition the knee in the target position.[18] For each lower limb, three repositioning attempts were performed. All procedures were conducted with the participants blindfolded, in order to eliminate visual inputs.

Repositioning errors were reported as: Absolute Angular Error (AAE), which is the absolute value of the difference between the value of the target range and the range reproduced by the participant;[19] Relative Angular Error (RAE), defined as the arithmetic difference between the value of the target range and the range reached by the subject[19] (negative RAEs indicate a directional bias into the extension movement, and positive RAEs signal a bias into the flexion movement); and Variable Angular Error (VAE), defined as the standard deviation of the three repositionings.[20]

Flexibility assessment

Hamstring flexibility was assessed through the sit and reach test. For this test, participants sat on the floor barefoot and with their feet set approximately hip-wide against a testing box, with their knees extended. Then, they were instructed to place one hand over the other, and slowly reach forward as far as they could by sliding their hands along the measuring tape, and to maintain the maximum position for 2 seconds.[21]

In order to understand how different levels of hamstring flexibility might influence the KJPS, after collecting all participants' data from the sit and reach test, two categories of flexibility were established (low and high), taking into account the median of this variable (17.40cm). Participants with a median flexibility equal or lower than 17.40cm were placed in the low flexibility category, and those with values above 17.40cm were placed in the high flexibility category.

Statistical procedures

Statistical data were analyzed using the Statistical Package for Social Sciences (SPSS) software (26.0 version). AAE, RAE, VAE and anthropometric variables (age, BMI) are described as Median and Interquartile Range (Med; IQR). The Shapiro-Wilk Test was applied to help understand the distribution characteristics of the data. The statistical significance of differences of medians between the flexibility categories and variables like age, BMI, AAE, RAE and VAE were verified using the independent-samples Mann-Whitney U Test. In order to check for possible associations between flexibility and repositioning errors, the Spearman Correlation Coefficient was also calculated. For all analyses, the level of significance was set at $p<0.05$.

Results

No significant differences were found between the flexibility categories regarding age ($p=0.579$) or BMI ($p=0.220$) (Table 1).

Table 1. Comparison between low and high flexibility categories regarding age and BMI.

	Low flexibility (n=21)	High flexibility (n=20)	*p*
	Med; IQR	Med; IQR	
Age (years)	21.00; 1.00	21.50; 2.00	0.579
BMI (kg/m^2)	23.89; 4.88	24.67; 7.27	0.220

Legend: BMI: Body Mass Index; IQR: Interquartile Range; Med: Median
Source: The authors (2022).

No significant differences were found between the flexibility categories and the AAE (p>0.05). Also, participants from both flexibility categories tended to overestimate the target position. However, the RAE was not significantly different between the categories (p>0.05). Similarly, the consistency between the three repositionings given by the VAE showed no significant difference between the low and high flexibility categories, both for the dominant as well as the non-dominant limb (p>0.05) (Table 2).

Table 2. Comparison between low and high flexibility categories regarding AAE, RAE and VAE of the dominant and non-dominant limb.

		Low flexibility (n=21)	High flexibility (n=20)	*p*
		Med; IQR	Med; IQR	
AAE	**DL**	2.41; 4.15	4.00; 3.78	0.566
	NDL	3.11; 3.83	3.73; 3.21	0.489
RAE	**DL**	-2.41; 4.15	-4.00; 3.78	0.566
	NDL	-3.11; 3.83	-3.73; 3.36	0.734
VAE	**DL**	1.63; 2.15	1.40; 1.52	0.948
	NDL	0.90; 1.32	1.45; 1.69	0.171

Legend: AAE: Absolute Angular Errors; DL: Dominant Limb; IQR: Interquartile Range; Med: Median; NDL: Non-dominant limb; RAE: Relative Angular Errors; VAE: Variable Angular Errors

Source: The authors (2022).

No associations were found between hamstring flexibility assessed through the sit and reach test and repositioning errors, in both the dominant and non-dominant limb (Table 3).

Table 3. Association between flexibility and the repositioning errors of the dominant and non-dominant limb.

		Flexibility	*p*
		Correlation Coefficient	
AAE	**DL**	0.079	0.623
	NDL	0.027	0.868
RAE	**DL**	-0.079	0.623
	NDL	0.013	0.935
VAE	**DL**	0.014	0.931
	NDL	0.230	0.212

Legend: AAE: Absolute Angular Errors; DL: Dominant Limb; NDL: Non-dominant limb; RAE: Relative Angular Errors; VAE: Variable Angular Errors

Source: The authors (2022).

Discussion

This study seeks to evaluate the influence of hamstring flexibility on the KJPS.

Although proprioceptive signals from both agonist and antagonist muscles around a given joint contribute to the sensation of limb position, it is argued that the information from the

muscles being stretched during the repositioning task is responsible for the most important contribution,[22,23] which can be attributed to the muscle spindle function.[6] According to this hypothesis, in a knee extension repositioning task, the hamstrings are expected to give the greatest contribution to the sense of position, since they are being stretched. Accordingly, different levels of flexibility of this muscle group may influence the KJPS. However, the results of the present study revealed no significant differences in repositioning errors between the low and high flexibility categories, suggesting that increased or diminished hamstring flexibility does not influence knee extension repositioning accuracy in the selected target position. In addition, no significant associations were found between flexibility and the repositioning errors.

To our knowledge, only the study of Akman and colleagues[15] has investigated the influence of hamstring flexibility on KJPS, through an analysis of both elite dancers and sedentary individuals. Although the flexibility assessment and the repositioning method were similar to those of the current study, three target ranges (20º, 40º and 60º of knee flexion) were tested. The authors analyzed the association between flexibility and active position sense, and reported significant negative correlations between these variables when repositioning to 20º. This finding is not aligned with our results, which do not suggest any positive or negative effect of hamstring flexibility on KJPS. Still, the conclusions of the study of Akman and colleagues[15] must be considered with caution since the authors concluded that higher values of flexibility negatively affected knee JPS in both dancers and sedentary participants. However, a negative correlation would mean that greater flexibility would lead to fewer repositioning errors, which represents a positive and not a negative effect on proprioceptive acuity that the authors failed to recognize. It is important to note that the results of Akman and colleagues[15] were especially noticeable when repositioning to 20º flexion. Repositioning to 40º flexion, a target similar to that of the present study, also revealed a significant negative association, although only in the subgroup of dancers.

With regard to directional bias, the results of the current study also failed to reveal differences between the flexibility categories, despite the probability of participants with lower hamstring stretching capacity sensing their legs in a more stretched position than actually happened (underestimation of the target position) and vice versa. However, the lack of studies on this topic does not allow these assumptions to be related to another research. Similarly, the consistency of repositionings was similar between participants with low and high flexibility. Nevertheless, as already stated, no articles with this assessment have been conducted in the past to confirm or refute these results.

Some limitations of the study should be recognized. First, the sample size was relatively small. Second, the chosen target position was not a range that required a significant stretching of the hamstring and, consequently, of the muscle spindle. This may explain the absence of differences between individuals with greater and lesser flexibility. However, according to the work of Olsson and colleagues[20] on the knee joint, muscle mechanoreceptors are more active in intermediate ranges, specially between 40º and 80º of knee flexion, and the chosen target position for this study was a range that lies within this interval. Moreover, only repositioning to extension was assessed, and flexion repositioning tasks could have provided additional relevant information. Third, the fact that the chosen test to assess hamstring flexibility was the sit and reach test, which, according to Mayorga-Vega and colleagues,[24] has a moderate mean criterion-related validity for estimating hamstring extensibility. Fourth, information regarding the menstrual cycle phase of female participants was not collected. According to Miyazaki and colleagues[25] the menstrual cycle has implications for flexibility measurement, since passive

stiffness is significantly decreased during the ovulatory phase when compared with the follicular phase. In addition, Fouladi and colleagues[26] reported that female athletes have varying levels of KJPS during the menstrual cycle, with worse JPS at menses and greater accuracy during the mid-luteal phase.

Conclusion

These findings imply that higher or lower hamstring flexibility does not influence knee repositioning accuracy to extension, suggesting that having lower or higher flexibility does not impair the KJPS.

Further research on this topic should be conducted with more robust samples in order to confirm or refute the results presented in this paper, especially regarding directional bias between different levels of hamstring flexibility.

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors state that no conflict of interest exists. No author has any financial interest in or has derived any financial benefit from this research.

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Medicinal plants vs. conventional medicine: treatment assessments for indigenous populations

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Abstract

Introduction: Although traditional healing practices are important for indigenous populations, access to modern health care practices is essential to ensure the health of communities as well as the prevention of diseases and their aggravation. Objective: To examine the acceptance of medication by the Pitaguary indigenous group, who live in Maracanaú, state of Ceará (CE), Brazil. Methodology: An observational and descriptive case of the use of natural plants as a medicinal treatment in the Pitaguary indigenous group, in Maracanaú-CE. The interviews used a questionnaire adapted from the SATIS-BR scale. Results: The majority of the Pitaguary population in the interview presented chronic diseases (diabetes or hypertension). Only 13.3% do not follow up with their community medical doctor. Of the interviewees, 20% use a mix of conventional and herbal treatments; 67.2% use a non-secure method to identify medication; and 52% suspend use of conventional drugs in the middle of treatment and replace them with herbal plants recommended by the village shaman. Discussion: The main strategy when working with indigenous populations ends up being health education about the use of medicinal plants in association with commercial drugs. The involvement of community's leaders in the delivery of health services seems an effective strategy to promote their understanding about dual use of medicinal plants and western drugs for chronic diseases. Conclusion: A culturally sensitive approach that takes into account indigenous traditions and beliefs should be used to improve medication adherence within indigenous groups.

Keywords: Indigenous, Conventional, Medicinal plant, Pitaguary.

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BJHBS, Rio de Janeiro, 2023;22(2):98-105
DOI: 10.12957/bjhbs.2023.80040
Received on 26/08/2023. Approved on 19/10/2023.

Introduction

The use of medicine by indigenous groups varies according to culture and tradition. Many indigenous communities have traditional healing knowledge and practices that include the use of medicinal plants, ritualistic practices, and natural medication.[1] However, these practices are often threatened by the access to adequate medical drugs and health care, in addition to pressure from Western medicine and the sale of industrialized drugs.[2]

Traditional healing practices and the use of medicinal plants are a fundamental part of the culture and traditions of indigenous populations. These practices are passed down from generation to generation and play an important role in community identity and cohesion.[3] Furthermore, the preservation of cultural traditions and healing practices is also important to ensure the emotional and spiritual welfare of indigenous tribes. Many indigenous communities employ a holistic approach to health that includes connection to the land, spirituality, and social relationships. Therefore, the preservation of these practices is essential to ensure the health and well-being of indigenous populations.[4]

Although traditional healing practices are important for indigenous tribes, access to modern health care practices is essential to ensure the health of the community and the prevention of diseases and their aggravation. Indigenous communities often have difficulty in accessing health care, including medicine, vaccines, and medical treatments. As a result, many indigenous people face serious chronic health problems, such as diabetes, heart disease and tuberculosis.[2]

Indigenous tribes have the right to receive culturally appropriate health care and their practices and beliefs must be respected. Therefore, health policies must be developed in partnership with indigenous communities, in order to guarantee access to appropriate medicine and health treatments, and conventional medication while ensuring respect for their beliefs, traditions and cultures.[3,5]

Usage of conventional medicine can be effective in preventing and treating diseases in indigenous communities. In this context, this work aims to evaluate the acceptance of conventional medication by indigenous communities, especially those of the Pitaguary people, who live in Maracanaú, in the state of Ceará (CE) in northeast Brazil, by addressing issues related to the preservation of culture and traditions, access to health care, and challenges faced by communities in the use of modern medicine, especially possible interactions between their natural plants and conventional drugs.

Methodology

This is a prospective, observational, and descriptive case-control study of the Pitaguary indigenous people, who live in Maracanaú. Located in Maracanaú and Pacatuba, in the state of Ceará, the Pitaguary indigenous territory is populated by four indigenous groupings: Olho d'água, Horto, Santo Antônio and Munguba. Maracanaú is a small town located in the metropolitan area of the city of Fortaleza, and the Pitaguary communities are part of its population. According to the Special Department for Indigenous Health, the Pitaguary tribe comprises 2,881 indigenous people divided into 4 groups.

The research took place between September 2017 and January 2018 and was approved by the human research ethics committee of National commission of Ethics and Research – Brazil (CONEP) under number 1.331.534. All research complies with resolution 196/96; a free and informed consent form was signed by each family; and the personal data of the participants were kept confidential. The indigenous population interviewed in this study were 243 families living in the Pitaguary communities of 400 index families in National Foundation of Indigenous People (FUNAI).

Community health workers and researchers collected data using a questionnaire adapted from the SATIS-BR mental health satisfaction scale by visiting each family to explore the commu-

nity's cultural and sociodemographic profile, the use of medicinal plants and the influence of these traditions on treatment adherence in the handling of chronic diseases. Interviewees must be of legal age, sign an informed consent form and live with their family in the indigenous territory. Individuals who did not meet these criteria were excluded from the survey.

The results were calculated based on the information provided by the population through the questionnaire. Tables were compiled with information on the prevalence of chronic diseases in the families interviewed.

Results

Presence of chronic diseases in the Pitaguary community of Maracanaú/CE

In total, 207 families from the Pitaguary indigenous community were evaluated. According to Graph 1, 60% of the population claim to have at least one chronic disease, the most prevalent of which are diabetes mellitus and chronic hypertension (Figure 1).

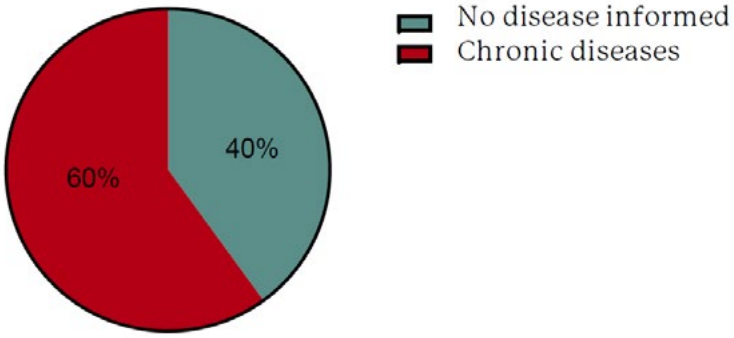

Figure 1. Presence of chronic diseases in the Community Pitaguary-Maracanaú/CE
Source: The authors (2023).

Adherence to follow-up by the family health team in the Pitaguary communities of Maracanaú/CE

Based on Figure 1, we questioned families about the consistency of their treatment for chronic diseases with the recommendations of the health teams assigned to the village. The results showed that 13.3% of the families interviewed did not report doing any follow-up. Of the 86.7% who were being followed up by the family health team, 20% did not use the appropriate drug therapy prescribed by the family doctor to treat chronic pathologies and 18.3% stopped the treatment after concluding by themselves that it was unnecessary or that their health condition had improved. Another important reason cited by the population for discontinuing medication was the difficulties in administering many drugs at the same time (Figure 2).

Education on the correct identification of medication for treatment of chronic disease

Considering that one of the reasons why families report difficulties in maintaining drug therapy is related to the volume of medication being administered, the study examined possible difficulties in the correct identification of medications, which increase problems with medication adherence. The most mentioned ways of identifying medication were by the name on the

packaging 32.8% use the most secure identification mode considered: identifying medication by the name (Figure 3). And 67.2% of the families interviewed use insecure methods of identifying the right medication and the correct time of administration, such as trusting only where the box of medication is located at home, color, or size of the medication without checking the name of the medication. It is interesting to note that most interviewees informed that they were able to buy the medication if it were not available for free through the SUS (Brazilian Unified Health System). However, 10% are completely dependent on the SUS and this share of the population will not have a good treatment condition for any pathology in case of impediments to access. This last piece of information is a timely reminder of the importance of keeping the SUS in full operation, in order to avoid impairing the treatment of the indigenous population.

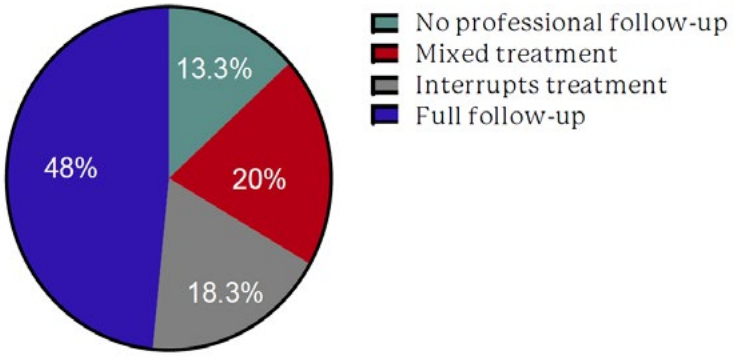

Figure 2. Adherence to follow-up by the family health team in the Pitaguary's communities, Maracanaú/CE
Source: The authors (2023).

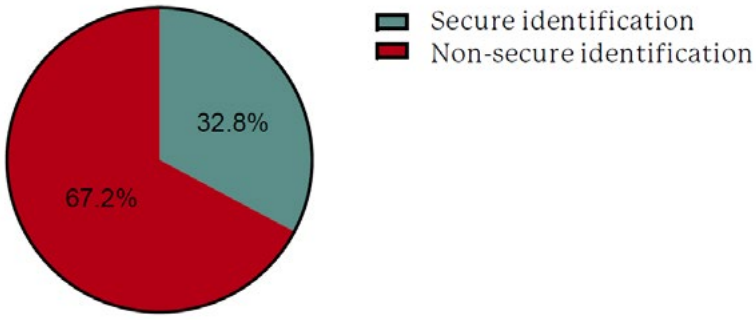

Figure 3. Education on the correct identification of medication for the treatment of chronic disease
Source: The authors (2023).

Dual use of conventional drugs and herbal medicines in the Pitaguary communities of Maracanaú/CE

When evaluating whether this community use both medications, conventional drugs prescribed by doctors with medicinal plants (herbal medicines) available in the community, 35.2% claim to do the treatment with dual use and 52% even report suspending the use of conventional drugs in order to use only herbal medicines recommended by the village shaman (Figure 4). All the interviewees state that the use of herbal medicines is not accompanied by any side effects, which facilitates acceptance by the community, while 60% of the

population report side effects when using conventional drugs. This conclusion leads the community to be more wary of using medication prescribed by family doctors for the treatment of acute or chronic diseases.

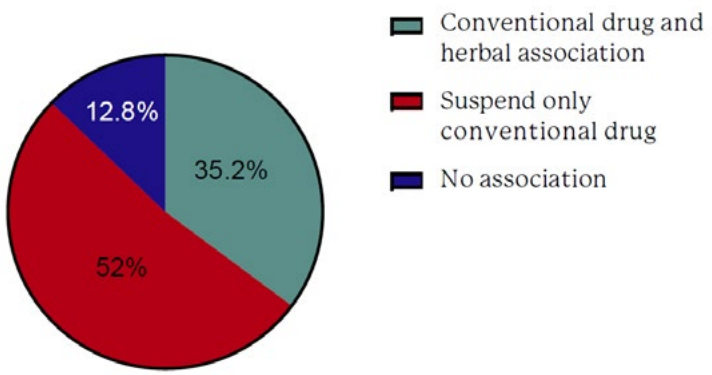

Figure 4. Association between conventional drugs and herbal medicines in the Pitaguary's Communities, Maracanaú/CE

Source: The authors (2023).

Discussion

This study showed the presence of chronic diseases in an indigenous Pitaguary population in Maracanau/CE. A population that showed values comprehensive care, an increased risk in the secure identification of medication for the treatment of chronic diseases and a predominance of the use for natural medicines present in the community, putting at risk treatment with conventional drugs already tested and prescribed by the doctor. In a review of the literature on indigenous populations and chronic diseases, Umaefulam and colleagues[6] reported a strong presence of chronic diseases, such as diabetes and hypertension, but when evaluated the percentage of indigenous group enroled for clinical studies from United States of America (USA), Canada, New Zeeland and Australia, only 5.6% of the individuals enrolled were the indigenous, showing how is still difficult to address these population to clinical care. In addition, a study published by the School of Medicine, Missouri, USA, in 2021,[7] identified a high prevalence of cardiovascular diseases in the Native American population. Our study corroborates findings in the literature that show indigenous populations with a high incidence of chronic diseases and a need for quality health care.

Medication adherence, which is the patient's ability to correctly follow medical guidelines regarding the use of medication, is a problem faced by many people, including indigenous populations,[8] which needs to be addressed and corrected. Adherence to herbal medication among the indigenous population can be driven by a variety of factors, including the availability of local indigenous medicinal plants, culture and traditions related to the use of herbs and plants, accessibility and availability of allopathic medicines, and health education passed on by previous generations.[9]

Many indigenous communities have a robust tradition of using local medicinal plants to treat a variety of ailments; however, the introduction of so-called conventional drugs often leads to a decline in the use of medicinal plants, which can have negative effects

on the preservation of culture and tradition.[10] Furthermore, the availability of conventional medicines may be limited within many indigenous communities, especially in remote areas, which can lead to a greater dependence on medicinal plants. Nevertheless, no evidence exists that such plants are effective in combating diseases such as diabetes and high blood pressure.[11] Many indigenous communities have faced histories of trauma and violence, including colonization, genocide, displacement, and marginalization. This can lead to distrust and resentment towards health professionals, who may be seen as agents of the State and the dominant culture.[12]

Therefore, adherence to herbal medication among the indigenous population is a complex and multifactorial problem that reflects a variety of influences, including the availability of medicinal plants in the vicinity, indigenous culture and traditions related to the use of herbs and plants, accessibility and availability of herbal medicines and health education.[13]

In this study, the Pitaguary population exhibited difficulties in safely identifying medication. Health education also plays an important role in the acceptance of herbal remedies, since the culture and customs of a community can influence the way in which people perceive and use medication.[14] Some groups may distrust modern medicines or lack access to them. Therefore, health professionals should establish effective means of communication with the communities served, in order to understand their needs and to assess difficulties better; thus, being able to offer guidance and competent care.[15]

To deal with such challenges, health professionals must respect and value traditional knowledge and practices of indigenous medicine by listening and learning from community members. These methods can also be complementary and integrate into a more holistic approach and integrative health. In a study with the same population in 2011,[16] a pilot project was implemented by multidisciplinary students from the State University of Ceará (UECE) in partnership with the PET indigenous health project (organized by the SUS). This pilot project developed stickers that provide guidance on the correct times to take medication, allowing patients to monitor the use of medication and to observe improvements in chronic pathological conditions. In addition, the pilot project showed a need for professional training so that professionals can understand the culture, languages, and values of these communities, in order to establish relationships of trust and mutual respect.[17]

The main strategy when working with indigenous populations ends up being health education and the use of medicinal plants in association with commercial drugs. As observed in this research, 52% of the population stopped using medication prescribed by the family health team and opted to rely only on the use of medicinal plants. The uses of plants for the indigenous population in other continents of the world have a strong prevalence of medicinal use, in alignment with the results found in the Pitaguary population.[18,19]

The involvement of community leaders and elders in the delivery of health services so that indigenous health practices are valued and respected is usually an effective strategy. In addition, indigenous health practices, including the use of medicinal plants, should be recognized and promoted, so that the community feels more comfortable and confident in adhering to the correct treatment for chronic diseases.[20] Risks and possible side effects should be emphasized, so that the community is aware of the consequences of decisions involving the suspension of drugs or the dual use of conventional drugs and herbal medicine among them.[21]

Conclusion

In order to improve compliance among indigenous groups and to achieve benefits from greater control of chronic diseases through the safe identification of medication and follow-up contacts with the health team, a culturally sensitive approach must be adopted that takes into account traditions and beliefs. In addition to guaranteed access to medicine in an adequate and regular manner, clear and accessible information about the use of medicines and their side effects should be provided, Moreover, community leaders and local authorities must be involved in the process of raising awareness about the importance of medication adherence and in promoting health practices that are more appropriate to the reality of indigenous communities.

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BJHBS

Prevalence of vitamin D deficiency in children with sickle cell anemia: a systematic review

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Abstract

Sickle cell anemia is a genetic disease that is highly prevalent in the Brazilian population, especially among black ethnic groups descended from migration of enslaved people from Africa, as well as descendants from the process of miscegenation. The disease is a clinical expression of the homozygosity of the hemoglobin S gene, which may be of genetic and/or hereditary origin, and is caused by the replacement of the normal residue of glutamic acid with the amino acid valine in the sixth position of the polypeptide chains of the beta-globin protein. This process generates biochemical alterations in hemoglobin S molecules that polymerize inside the erythrocyte and transform into sickle cells. Lack of vitamin D may possibly be linked to disease processes, as well as to individuals' pain crises. Objectives: To conduct a systematic review in order to analyze the prevalence of vitamin D deficiency in children with sickle cell anemia. Methodology: A systematic review was carried out by means of a search for original articles in the English and Portuguese languages in the following scientific databases: Pubmed, Science Direct, Lilacs and Scielo. Results: Among 10 articles found, 8 showed a prevalence of vitamin D deficiency (<20 ng/mL) in children with sickle cell anemia. Conclusion: The prevalence of vitamin D deficiency was high in patients with sickle cell disease, so supplementation of this substance may be helpful in treatments to improve the condition and in preventing deficiency. However, further studies that target this association and/or intervention are required to obtain more practical results.

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BJHBS, Rio de Janeiro, 2023;22(2):106-116
DOI: 10.12957/bjhbs.2023.80047
Received on 23/01/2023. Approved on 20/09/2023.

Keywords: Sickle Cell Anemia; Nutritional Status; Children; Vitamin D.

Introduction

The identification of nutritional status through diagnostic procedures is essential for the diagnosis of possible nutritional disorders of individuals and/or the population, in addition to the establishment of degrees of risk, as well as causal and nutritional factors.[1]

According to Mataratzis and colleagues,[2] sickle cell anemia (SCA) is characterized by a prevalent homozygous form of hemoglobin S, that is, it is characteristic of individuals who receive an abnormal hemoglobin S gene from the father and another from the mother. To be considered a trait anemia, the patient must receive a gene for normal hemoglobin (A) and another for abnormal hemoglobin (S), thus becoming a carrier of sickle cell anemia trait (AS). In Brazil, this disease varies by region, with higher rates in the Northeast, due to a high prevalence of descendants of Africans. On average, 3,500 children are estimated to be born with sickle cell disease every year in Brazil, to such an extent that it is considered a public health problem.

Approximately 5% of people in the world carry genes responsible for hemoglobinopathies in their structure, with an estimated 5,476,407 children born with sickle cell trait (SA) and 312,302 with HbSS worldwide every year. In underdeveloped countries, such as those of sub-Saharan Africa, the estimated number of live births with the HbSS gene is 235,681 births per year. This number is much higher than in developed countries, such as the United Kingdom, which has a rate of 300 births; and the United States of America (USA), with approximately 3,000 births.[3]

Patients with sickle cell anemia are initially asymptomatic in the first six months of life due to the presence of fetal hemoglobin (HbF), whose concentrations are higher than those of adults. Even so, this pathology is associated in childhood with high incidences of morbidity and mortality due to sepsis, splenic sequestration, aplastic crisis, acute chest syndrome and stroke.[4]

According to Hankins,[5] chances of survival can be increased in children exposed, at an early stage, to simple measures: early diagnosis, anticipatory guidance, prophylactic treatment and implementation of neonatal screening programs, including neonatal screening and preventive pediatrics, linked to genetics.[6]

Since the disease is transmitted through a genetic factor, genetic counseling is considered to be of crucial importance, in order to provide guidance both to patients about traits of the disease as well as to those in which the disease is already developed. Counseling helps to enhance the reproducibility and understanding of certain aspects of the disease, such as suffering, treatment and prognosis. This genetic counseling is of an auxiliary nature, providing guidance to individuals and families of individuals with the pathology. For the purposes of this counseling, it is necessary to establish whether the patient is a homozygote or a heterozygote, and hemoglobin S must be confirmed and differentiated from other hemoglobins.[7]

Salles and colleagues[8] state that children with sickle cell anemia may develop airway obstructions and even hypertrophy, with a prevalence of 55.3% of obstruction being observed. This condition may be associated with obstructive sleep apnea syndrome as well as contribute to episodes of hypoxemia. One of the most important characteristics of the disease is the vaso-occlusive crisis, also known as sickle cell crisis, resulting in the obstruction of small blood vessels, tissue hypoxia, necrosis, and severe pain. Strokes and chronic hemolytic anemia are also common.

Sickle cell anemia is a chronic and incurable disease that is amenable to treatment, but even so, inflicts a great degree of suffering on its carriers, who merit special attention from the den-

tal, medical, psychosocial and genetic points of view. The obstruction of blood vessels generates pain crises, with swelling and necrosis in various organs, such as bones and joints, spleen, lungs and kidneys.[9]

According to studies, calcium and vitamin D are involved in the bone metabolism, such that low calcium intake leads to a reduction in the ideal bone mass peak among children and adolescents, constituting an aggravating or determining factor of impaired growth in children and adolescents with sickle cell anemia. Deficiencies in these substances lead to fragility and bone deformities, such as rickets, which is a classic condition derived from the lack of these micronutrients, according to studies that have examined dietary inadequacy.[2]

Vitamin D

According to Castro,[9] the term vitamin D encompasses a group of secosteroid molecules derived from 7-dehydrocholesterol (7-DHC) that is interconnected through various photolytic and enzymatic reactions occurring in cells of different tissues. These substances thus cover both active metabolisms (1 to, 25-dihydroxyvitamin D or calcitriol) and their precursors (vitamin D3 or cholecalciferol, vitamin D2 or ergosterol and 25-hydroxyvitamin D or calcidiol), as well as products of their degradation, which may still maintain some metabolic activity.

In humans, only 10 to 20% of the necessary vitamin D comes from our diet. The main dietary sources are vitamin D3 (cholecalciferol, of animal origin), which is present in fatty fish from cold and deep waters, such as tuna and salmon, and vitamin D2 (ergosterol of vegetable origin), which is present in edible fungi. The remaining 80% to 90% is synthesized endogenously.[10]

According to Alves and colleagues,[11] the role of vitamin D is to regulate the phosphocalcic metabolism, in order to ensure bone mineralization functions. It is the only vitamin that can be synthesized by the skin from exposure to sunlight (ultraviolet radiation).

The vitamin obtained from ultraviolet irradiation is D3, as sunlight hits the skin and forms pro-vitamin D3, which in contact with the skin is transformed into pre-vitamin D3. Both D2 and D3 can also come from the diet. In the metabolism, the substance subsequently undergoes hydroxylation in the liver by 25-hydroxylase and becomes 25-hydroxyvitamin D. This process requires additional hydroxylation in the kidney by 1 alpha-hydroxylase to then form the biologically active form of vitamin D 1,25-dihydroxyvitamin D.[11]

According to Nolan and colleagues,[12] vitamin D (25-hydroxyvitamin D) deficiency has emerged as a public health issue in recent years due to its contribution to skeletal and extra-skeletal manifestations. Individuals with sickle cell disease reportedly tend to present a high prevalence of vitamin D deficiency.

Among the vitamins, according to Oliveira and colleagues,[13] vitamin D should be carefully evaluated in children and adolescents with sickle cell anemia, since low amounts occur due to the high concentration of melanin in the skin, reduced levels of physical activity and low food intake, all of which contribute to the development of the deficiency.

According to Soe and colleagues,[14] the increase in catabolism can generate an energy deficit and even nutrient absorption, so that those who present this pathology can suffer from multiple deficiencies of macronutrients and micronutrients. Among these is vitamin D, which is responsible for calcium homeostasis and also essential for bone mineralization, such that vitamin D deficiency may harm skeletal muscles.

According to Oliveira and colleagues,[13] for Dietary Reference Intakes, vitamin D deficiency occurs when the serum concentration of 25-hydroxyvitamin D is lower than 11ng/mL, and is possibly also related to the pain crises of individuals with the sickle cell disease, although no studies have been able to link the two phenomena.

Still, according to Alves and colleagues,[11] when a sufficient amount of vitamin D is present, more phosphorus and calcium is absorbed, so that osteoblasts can use 1,25-dihydroxyvitamin for D to interact with the vitamin D receptor, inducing immature monocytes to become mature osteoclasts and dissolve the matrix, thereby fixing calcium and other skeletal minerals.

According to Adegoke and colleagues,[15] vitamin D (D3) supplementation can serve as an anti-inflammatory substance in the treatment of sickle cell disease. However, they consider the number of studies on the subject to be somewhat low, since some articles about children refer to an extra supplementation of vitamin D3 of 2000 IU per day, over a period of three months, thus generating an increase in cytokines.

In addition, Arya and colleagues,[16] in a case report study that associates chronic pain with sickle cell disease, showed that vitamin D supplementation through intramuscular injection of 600,000 IU (15 mg) of cholecalciferol (vitamin D3) can be suitable for the relief of chronic pain, subject to accompanying laboratory tests.

Brazil has high rates of sickle cell anemia, especially among individuals of African descent, leading the disease to be considered a public health problem.

Nutritional status is known to be a major aggravating factor for the disease, and must be taken into consideration during treatment. Vitamin D is one of the vitamins that may be associated with this pathology, thus presenting possible deficiencies, and may be correlated with pain crises in individuals if it is not ingested in correct amounts.

In light of these considerations, the present study seeks to analyze the prevalence of vitamin D deficiency in children with sickle cell anemia, through a systematic review, in order to shed light on possible associations between the two conditions.

Methodology

A systematic review was carried out by means of a search for original articles in English and Portuguese, published during the last 12 years. The search sought to identify the prevalence of vitamin D deficiency in children with sickle cell anemia. The scientific databases used were: Science direct, Pubmed, Medline, Scielo and Lilacs. Scientific journals and journals available in electronic format were also used.

The descriptors used in Portuguese and in English were: “sickle cell anemia”, “nutritional status of children with sickle cell anemia”, “sickle cell anemia as public health problem”, “prevention and sickle cell anemia”, “symptoms and sickle cell anemia”, “treatment and sickle cell anemia”, “vitamin D”, “vitamin D and sickle cell anemia”, “vitamin D deficiency in children with sickle cell anemia”, “prevalence of vitamin D deficiency in children with sickle cell anemia”, and “vitamin D deficiencies in sickle cell anemia.”

The inclusion criteria for conducting the systematic review were initially a search for articles according to the titles and abstracts analyzed. The search focused on studies that dealt with children while also presenting the amounts of vitamin D deficiency among the participants,

thus showing possible associations with the pathology of sickle cell anemia. After an electronic search, priority was given to the most recent publications.

Research unrelated to the topic, literature reviews, and animal studies were excluded, and a critical analysis was performed of each of the selected studies, in order to assess the validity of the results obtained and the possibility of conclusions being based on correlated data.

Results

Table 1 presents the articles selected for the present study, describing author, year, location; sample number; mean age; vitamin D dosage; p-value; duration and type of study; and adequacy of vitamin D deficiency in relation to the established dosage.

Table 1. Data collection of children with sickle cell anemia x adequacy and/or inadequacy of vitamin D.

Author, year and place	Sample size	Mean age	Vitamin D dosage	*P***	Duration and type of study	Results of vitamin D deficiencies
Adegoke et al, 2017 (Nigeria)[15]	170	7 years	>=30ng/mL	*P*>0.05	12 months Transversal	Negative
AlJama et al, 2018 (Saudi Arabia)[17]	640	12 years	<20ng/mL	*P*<0.05	5 years Transversal	Positive
Jackson et al, 2012 (USA)[18]	139	11.5 years	<20ng/mL	*P*<0.05	4 years Transversal	Positive
Rovner et at, 2008 (USA)[19]	150	11.5 years	<20ng/mL	*P*<0.05	1 year Student's T-Test	Positive
Wykes et al, 2014 (London)[20]	81	9,8 years	<20ng/mL	*P*<0.05	NR	Positive
Garrido et al, 2012 (Spain)[21]	78	4.3 years	<20ng/mL	*P*<0.05	3 years Transversal	Positive
Adegoke et al, 2017 (Nigeria)[22]	123	8 years	>=30ng/mL	*P*>0.05	NR Transversal	Negative
Adegoke et al, 2016 (Brazil)[23]	36	7.5 years	<20ng/mL	*P*<0.05	NR Cross-sectional Prospective	Positive
Lee et al, 2015 (Colombia)[24]	95	10.6 years	<20ng/mL	*P*<0.05	1 year e 4 months Transversal	Positive
Osunkwo et al, 2011 (NR)[25]	53	12 years	<20ng/mL	NR	2 years NR	Positive

Legend: NR: Not Reported.
Source: The authors (2022).

According to the study by Adegoke and colleagues[2] carried out in Ile-Ife, Nigeria, using data collected from 170 patients over a period of twelve months in children with a mean age of 7 years, 2 of whom were diagnosed with vitamin D deficiency (<20ng/mL), 12 with insufficiency (<30ng/mL) and 156 with sufficient levels (>=30ng/mL), while 95 of these children had sickle cell anemia in a steady state, that is, the period during which the child is free from pain, infection and/or any other illness, while 75 children were apparently healthy.

The result of this experiment established that the means of both groups were similar and the serum pro-inflammatory cytokines measured during the study of children with suboptimal vitamin D (<30ng/mL) were significantly higher than those in children with normal serum vitamin D (>=30ng/mL), such that the cytokines showed increased values in children with sickle cell anemia compared to healthy ones. Although the study found two children diagnosed with deficiency and 12 with insufficiency, most children obtained adequate levels of vitamin D, indicating a negative result for vitamin D deficiency.[2]

Another study, by AlJama and colleagues,[17] was carried out over a period of 5 years in the Eastern Province of Saudi Arabia, in the city of Al-Qatif, in which 640 children were monitored, with a mean age of 12 years, and vitamin D deficiency being confirmed by hemoglobin electrophoresis, independently of the status of the disease, through the performance of a vitamin D reading. The 49 children with sickle cell anemia had 25-OH-D levels of 16.3ng/mL, 463 children with sickle cell anemia in crisis periods had levels of 10.1ng/mL and 128 children without seizures had levels of 15.7ng/mL, which are considered to be extremely low amounts of vitamin D.

As a result, the mean level of 25-OH-D was statistically higher in the group of patients diagnosed with sickle cell anemia in pain crisis, but vitamin D levels were insufficient and deficient in all groups, in such a way as to present values of inadequacy. The study thus demonstrated a positive relationship between vitamin D deficiency with sickle cell anemia in children.[17]

In addition, Jackson and colleagues[16] carried out another study with 139 children diagnosed with sickle cell disease, with a mean age of 11.5 years, which evaluated seasonal issues related to vitamin D levels. The results showed that 96.4% of the children had vitamin D deficiency (<20ng/mL), 64% had severe deficiency (<10ng/mL), the levels of 1.4% were insufficient (20.01–29.9ng/mL) and 2.2% showed sufficient (>/30ng/mL) vitamin D. Therefore, the analysis of the results confirmed the pertinent vitamin D deficiency, although full multivariate models did not find significant associations of severe vitamin D deficiency (<10ng/mL) with episodes of pain attacks.

In the same line of study and using Student's T Test, Rovner and colleagues[19] analyzed 150 children diagnosed with sickle cell disease, with a mean age equal to that of the previous study. Of the individuals analyzed, 61 African-Americans with the condition had deficient serum 25-OH-D levels (15ng/mL) and 89 healthy African-American control subjects had insufficient serum levels (21ng/mL). The study found that vitamin D deficiency was 5.3 times higher in children with sickle cell anemia, signaling a positive association.

In addition, Wykes and colleagues[20] analyzed the prevalence of vitamin D deficiency and its pathophysiological correlates in 81 children with a median age of 9.8 years, through measurement of 25-OH-D by blood tests. Of the children participating in the study, only 1 had sufficient 25-OH-D (>=30ng/mL), 6 had insufficient levels (21-29ng/mL) and 74 showed deficiency (<20ng/mL). As a result, the widespread presence of vitamin D deficiency was confirmed. The age of the children was significantly correlated with deficiency levels, tending to decline as age increased, with a correlation between 25-OH-D levels and serum calcium levels.

In the study by Garrido and colleagues,[21] 78 African, African-American and Asian children, with a mean age of 4.3 years, diagnosed with sickle cell anemia and vitamin D deficient status, were analyzed in a 3-year cross-sectional study. Of the children analyzed, 56% had vitamin D deficiency values of 25(OH)D below 20ng/mL, with this percentage increasing to 79.5% when

considering 25(OH)D levels below 30ng/mL as insufficient, while 17.9% of individuals had 25(OH)D levels below <11ng/mL. The study found a high prevalence of insufficient and deficient levels of vitamin D in patients with sickle cell anemia.

In addition, Adegoke and colleagues[22] carried out a study with 123 children diagnosed with sickle cell disease, in order to verify the influence of serum 25-OH-D on episodes of acute pain in Nigerian children with a median age of 8 years. The study found that 14 children had deficient 25-OH-D levels (<20ng/mL), while 109 had sufficient levels (>=30 ng/mL), and none of the children presented severe vitamin D deficiency. Ninety children with sickle cell disease had at least one significant pain crisis, such that the frequency of pain was inversely correlated with the serum level of 25-OH-D, although this result was not statistically significant.

In another prospective cross-sectional study, Adegoke and colleagues[23] analyzed 36 children with a mean age of 7.5 years, under treatment at the Pediatric Hematology Clinic at the Federal University of São Paulo, diagnosed with sickle cell anemia and probable vitamin D deficiency associated with hemolysis biomarkers. Of the participants, 23 had sufficient levels of vitamin D (>=30ng/mL) and 13 had deficient levels (<20ng/mL). In this context, analysis of the relationship between serum vitamin D and hemolysis biomarkers showed that the mean hemoglobin of children with vitamin D deficiency was significantly lower than those without vitamin D deficiency. Therefore, this study can be considered positive in the face of vitamin D deficiency in patients with sickle cell disease, since the sample is small and a little less than half of the sample showed values below insufficient and sufficient.

The study by Lee and colleagues,[24] carried out in Colombia with 95 children with median age of 10.6 years, analyzed sickle cell anemia, vitamin D deficiency and acute vaso-occlusive complications over a period of 1 year and 4 months. The participants included 56 vitamin D deficient patients (<20ng/mL), 27 of whom were severely deficient (<10 ng/mL) and the status of 12 others was not reported. The result of this study was positive for vitamin D deficiency, since the mean level of 25-OH-D was 18ng/mL, with the lowest mean in spring (15.2ng/mL) and highest in summer (22.4ng/mL). However, overall, no significant differences were found across seasons. Significant associations made with the level of 25-OH-D and acute vaso-occlusive complications showed that 31 of these children had at least one episode of pain.

Osunkwo and colleagues[25] analyzed 53 children with mean age of 12 years, who had sickle cell disease, vitamin D deficiency and chronic pain. Of the participants, 15% had insufficient levels of vitamin D (21-29ng-mL), 32% were deficient (<20ng/mL), 40% severely deficient (<15ng/mL) and 13% profoundly deficient (<10ng/mL). A positive result of the study for vitamin D deficiency showed that 32% of children had the chronic pain and bone fragility associated with low levels of 25-OH-D.

Discussion

The term sickle cell disease (SCD) refers to a group of hereditary hemoglobinopathies resulting from a disorder in the morphophysiological morphology of hemoglobin (Hb). Among the types of DF, the genetic composition with the greatest clinical impact is sickle cell anemia disease (SCA), a condition in which the HbS gene is inherited from both parents and culminates in the homozygous form HbSS.[3]

The study presented by Samuel Ademola Adegoke and colleagues[2] demonstrated that the expression of cytokines with concomitant inflammation, cell adhesion to the vascular endotheli-

um and, consequently, endothelial injury, contributes to the process of vaso-occlusion in sickle cell disease. However, regarding the relationship between changes in cytokine levels and the pathophysiology of vaso-occlusive crisis, conclusions remain largely inconsistent.

This study also found that children with adequate levels of vitamin D (>=30ng/mL) had significantly higher levels of pro-inflammatory cytokines, with an inversely significant correlation with serum levels of 25-OH-D.[2]

This result stems from the fact that vitamin D has significant impacts on bone health and on the prevention of respiratory diseases, as well as hemolysis, thus contributing to the growing body of evidence about the preventive effects of vitamin D on immune disorders, since it triggers changes in the production of inflammatory cytokines and inhibits the proliferation of pro-inflammatory cells.[2]

These responses presented by vitamin D deficiency in patients with sickle cell disease are extremely important, since they result in bones being affected by microinfarcts, osteopenia, osteoporosis, osteomyelitis and osteonecrosis, as well as low bone mineral density. These conditions, as well as lack of exposure to sunlight, have been described in children with sickle cell anemia. Sunlight is considered essential for bone health, since approximately 90% of the necessary synthesis of vitamin D is derived from exposure to sunlight.[26]

Studies included in this review show that the onset of hypovitaminosis due to vitamin D in the general population of a sunny and dry country such as Saudi Arabia, for example, can approach 100%. This phenomenon leads to high rates of vitamin D deficiency in the population, insofar as to trigger a pandemic health problem, even in areas with sunshine all year round. This high incidence of vitamin D deficiency is more severe in children with sickle cell anemia in crisis. They are characterized by increased concentrations of inflammatory cytokines, which would explain the low levels of vitamin D during the sickle cell crisis, with symptoms of chronic pain observed both in cases of sickle cell disease and of vitamin D deficiency.[17] However, according to Alves and colleagues,[11] vitamin D levels can vary according to nutritional, genetic or even hormonal factors.

The high prevalence of severe vitamin D deficiency among children with sickle cell disease in different locations and latitudes may be related to cultural, behavioral, and environmental factors. Therefore, early screening for vitamin D deficiency is necessary to prevent bone mineral complications.[18]

Race, age, body mass index, latitude, diet, exposure to sunlight and skin pigmentation are all factors that influence the status of vitamin D. As well as its effects on bone health, vitamin D is associated with a variety of health conditions, including cardiovascular diseases, asthma, nephropathy and chronic pain.[12]

In addition, the concentrations of low vitamin D levels in 25-OH-D vary according to season and latitude. Its bioavailability, especially in winter, depends on diet and the body's hepatic reserves, and can lead to impaired intestinal absorption of vitamin D. Renal and liver abnormalities also play a role in vitamin D status in sickle cell disease.[27]

Another factor that may be significant for vitamin D production in sickle cell anemia is increased concentrations of melanin in the skin, particularly in African children, which is associated with reduced levels of physical activity and low vitamin D intake. Most African-Americans with sickle cell anemia tend to display this increase of melanin concentrations in the skin, so

they may not spend sufficient time outdoors to synthesize enough vitamin D, regardless of the geographic region in which they live.[19]

In light of the melanin concentrations, the nutritional factor undoubtedly influences the severity of the complications of the pathology , since the need for macro and micronutrients increases significantly due to the high metabolic demand caused especially by chronic hemolysis. The Basal Metabolic Rate (BMR) is 20% higher in children with sickle cell anemia compared to the population as a whole, because of increased catabolism.[28]

According to Wykes and colleagues,[20] vitamin D deficiency is considered common in sickle cell anemia, although the significance of its correction is not fully understood. Vitamin D is implicated in bone metabolism and in a wide range of physiological processes, as well as in the control of blood pressure, insulin secretion, lipid metabolism. Its deficiency is associated with the development of stroke, heart failure (HF), renal failure (RI), immunodeficiencies and even cancer. Severe deficiency in children causes rickets, osteomalacia with bone deformation, and may be associated with bone and muscle impairment, reduced growth and bone pain, so that low levels of the vitamin may result in a low absorption of sunlight, darkened skin, and food intake.

Mutational heterogeneity and genetic characteristics, as well as the environmental and social characteristics of individuals, are also factors that may be related to the clinical manifestations and complications of sickle cell disease.[29] According to Garrido and colleagues,[21] vitamin D deficiency is related to an increase in respiratory infections among children with sickle cell anemia and may also be related to increased skin pigmentation, reduced exposure to the sun and reduced intake of calcium and vitamin D. Vitamin D is highly important for bone health, but few articles analyze the risk of fractures in children with sickle cell anemia, so tests are necessary to assess the possible association between vitamin D levels and fractures.

Studies have shown that the serum 25-OH-D may influence the rate of significant episodes of pain in children with sickle cell anemia, suggesting a possible association between low levels of the serum and increased frequency of acute pain. Therefore, regular screening for vitamin D in children with suboptimal vitamin D levels is recommended, in conjunction with supplementation to reduce pain episodes.[22]

Another study, carried out by Samuel Ademola Adegoke and colleagues,[21] showed a correlation between decreases in hemoglobin and hematocrit levels after the reduction of serum 25-OH-D levels, suggesting a way in which vitamin D deficiency may play a role in the pathogenesis of the hemolytic phenotype of sickle cell disease. In patients with this pathology, hemolysis has been associated with reticulocytosis and endothelial dysfunction, triggering leg ulcers, pulmonary hypertension and stroke. Therefore, supplementation for these types of patients can be considered as standard procedure in order to improve health outcomes.

In addition, Martins30 has also shown that when the sickling of sickle cell anemia is formed, red blood cells begin to harden, and changes occur in membrane proteins and the expression of adhesion molecules increases. As a result, these red blood cells adhere to the endothelium, triggering an inflammatory phenomenon characterized by coagulation activation, hypoxia, ischemia, local infarction and reduced red blood cell survival. The repercussions of these alterations are responsible for the main signs and symptoms of sickle cell disease, such as pain, hemolytic anemia and progressive impairment of multiple organs, leading to morbidity and mortality.

Still according to Margaret and colleagues,[24] vitamin D deficiency is closely related to acute vaso-occlusive complications, which are associated with sickle cell disease in these individuals. These complications occur because patients with this pathology present a pro-inflammatory state with underlying microvascular obstruction and endothelial dysfunction, as well as vasculopathy induced by hemolysis and infections. Through its immunoregulatory and antimicrobial functions, vitamin D can help to control these processes and may help to reduce vaso-occlusive complications.

The study by Osunkwo and colleagues[25] showed that the peak effect on the reduction of days of pain occurred when serum levels of 25-OH-D exceeded 30ng/mL, indicating the possibility that vitamin D can also assist in pain reduction. Study findings confirm that individuals with sickle cell disease are particularly prone to vitamin D deficiency and suggest a link between this deficiency and bone fragility, demonstrating how vitamin D deficiency in children can lead to retarded growth and generate signs and classic symptoms of rickets, muscle weakness, which may also cause bone mineralization defects.[31]

Conclusion

This work aims to analyze, through systematic verification, the prevalence of vitamin D deficiency in children with sickle cell anemia, and concludes that the prevalence of vitamin D deficiency is high in patients with this pathology. Therefore, vitamin D supplementation may possibly serve as a means of treatment to improve the condition and prevent deficiency, and even decrease the frequency of pain crises. However, further clinical studies with larger sample sizes directly focused on this association and/or intervention are required.

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Beta-alanine supplementation and improvement of performance in swimming and water polo: a systematic review

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Abstract

Introduction: Beta-alanine is a non-essential amino acid that is naturally produced by the human body and used by many athletes and practitioners of physical activity to reduce muscle fatigue and to increase performance in high performance exercises. As a precursor of intramuscular carnosine, it has been shown to reduce the hydrogen ions that cause muscle fatigue during exercise in sports such as swimming and water polo. Objective: To evaluate various beta-alanine supplementation protocols in swimmers and water polo players and their effects on performance. Methodology: This study is a systematic review of various beta-alanine supplementation protocols used in exercises involving water polo and high-performance swimming, from searches on the Pubmed, SCIELO and RBNE platforms. Articles in English and Portuguese were included, which were evaluated on the basis of methods and parameters specific to swimming and water polo. Results: The review found that beta-alanine supplementation protocols varied according to the studies and their objectives. However, beta-alanine supplementation was shown to be effective in improving times, reducing blood lactate and increasing strength. Conclusion: Supplementation with beta-alanine generates a possible beneficial effect on swimmers and water polo players by fostering improvements in time, reduction of blood lactate and strength, all of which can positively influence the results of competitions and championships. The best protocols are those that administered a dose greater than or equal to 4.8g per day for, at least, four weeks.

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BJHBS, Rio de Janeiro, 2023;22(2):117-125
DOI: 10.12957/bjhbs.2023.80048
Received on 13/05/2023. Approved on 18/07/2023.

Keywords: Beta-alanine; Supplement; Carnosine; Swimming; Water polo.

Introduction

Beta-alanine supplementation has been the focus of studies in sports because its function, in conjunction with histidine, is to synthesize increased amounts of intramuscular carnosine. Studies show that increases in carnosine can occur through beta-alanine supplementation, which conclusion has been confirmed by a robust body of evidence produced since 2006, when the first study attesting to this fact in humans was published.[1]

Carnosine is a dipeptide found within skeletal muscle, whose function within the muscles is to buffer hydrogen ions, which are potentiators of muscle stress and acidosis, thus causing increased muscle fatigue during physical activity.[2]

According to studies, the properties of carnosine are related to its buffering effect, by acting directly in controlling muscle acidosis generated by hydrogen ions. Carnosine is formed by combining non-proteogenic amino acid beta-alanine with amino acid l-histidine (catalyzed by the enzyme carnosine synthetase) to form the dipeptide β-alanyl-L-histidine (i.e. carnosine). Once bound with beta-alanine, l-histidine is diverted from protein synthesis mechanisms, allowing high concentrations of this dipeptide to accumulate in skeletal muscle. Other factors that influence the amount of carnosine found include age, sex, type of muscle fiber, diet, and variables determining the concentrations of this compound.[2-4]

Administration of beta-alanine supplementation in powder form is associated with a rapid increase in the plasma amount at approximately 30-45 min after ingestion, regardless of the dose administered, which may be 10, 20, or 40mg/kg body weight. However, doses higher than 10mg/kg body weight are not well accepted. They generate discomfort in users, who complain of increased paresthesia, a neuropathy affecting the areas near the face, neck, shoulders, chest, and buttocks, usually in this exact order. Since paresthesia does not present health risks, this sensation can be considered a side effect rather than an adverse one.[5,6]

According to Matos,[3] the main characteristic of the effect of beta-alanine supplementation is a decrease in metabolic acidity, which stimulates an increase in carnosine concentrations and acts to improve the ability to perform acidotic efforts. This occurs due to the buffering effect of the hydrogen ions and their potentiation with histidine of a higher concentration of intramuscular carnosine, and has prompted athletes to increasingly seek its use. Its ergogenic function has been shown to be more effective within periods of high intensity and short duration, thus, studies have shown that its range lies between 30 seconds to 10 minutes.[3,7]

According to Maté-Muñoz,[8] the ingestion of high dosages of beta-alanine supplementation has always been correlated with a tingling sensation, manifesting itself commonly as irritability in the areas of the face, arms and hands. This effect happens because beta-alanine activates MrgprD receptors in primary sensory neurons, triggering an itching/tingling sensation that can last from 10 to 20 minutes, or even more than 60 minutes when taken in large doses. For this reason, the substance should be administered in several doses throughout the day in order to avoid adverse effects caused by the high dosages normally used when single doses are administered.

Beta-alanine supplementation is most recommended in exercises where the greatest use of energy and the main substrate comes from ATP, which occurs through anaerobic glycolysis, resulting in an increased release of lactic acid. The sports that most use the anaerobic glycolytic pathway include cycling, swimming, canoeing, water polo etc.[9]

Therefore, this study seeks to evaluate the effect of beta-alanine consumption on performance in water sports, and to describe the supplementation protocols used in conjunction with swimming and water polo.

Methodology

A systematic review was conducted using the Pubmed (Medline), Revista Brasileira de Nutrição Esportiva, and SCIELO databases. The descriptors used in the search and confirmed by the Descriptors in Health Sciences (DeCS) were beta-alanine, swimming, water polo, carnosine and supplementation, always in a combination with any of the five descriptors, using their equivalents in the English language.

The search was filtered for studies since 2012, and the inclusion criteria selected were original articles that address the theme of nutritional supplementation of beta-alanine in water sports, with a focus on swimming and water polo. Systematic review articles, meta-analyses, or those with possible conflicts of interest were excluded from reading and, consequently, from the review.

The inclusion criteria for the studies were the reading of titles and abstracts to exclude articles that did not study beta-alanine supplementation as a primary endpoint and that were available free of charge in the databases, while the exclusion criteria were the studies that did not fit the subject or were outside the field of nutrition.

The study was divided into three phases. The first followed the search criteria and the reading of abstracts and titles for analysis of the inclusion and exclusion criteria. The second comprised the full reading of articles for analysis of their effectiveness and descriptive contribution to the present study. The third phase was the development of the present article, its results and conclusions, in accordance with the criteria used in the two previous phases.

Results

In the 8 studies analyzed, a total of 164 male and female elite and amateur swimmers participated, 84% of which were men.

Most of the studies were conducted in a double-blind placebo-controlled fashion with the exception of the study by Mero,[10] in which both participants and researchers were aware that all subjects were consuming beta-alanine over a 4-week period (non-blinded). Two studies, by Painelli[11] and Mero[10] used the consumption of beta-alanine in conjunction with sodium bicarbonate as co-supplementation. In the study by Painelli,[11] two supplementation protocols were used, one solely with beta-alanine and one in conjunction with sodium bicarbonate, the latter being excluded from this survey. Mero,[10] meanwhile, analyzed only the joint supplementation of beta-alanine with sodium bicarbonate, so these results were maintained in our survey (Table 1).

The studies were separated into two groups according to their approach to diet and supplementation. The studies by Brisola,[12,13] Claus,[14] Norberto[15] and Mero[10] controlled the diet of the participants, while the studies by Chung[6] and Painelli[11] did not include any form of dietary control.

In the studies by Brisola,[12,13,16] the method adopted was the non-use of supplementation for at least three months prior to the study, as well the absence of dietary controls. Differently,

Claus[14] maintained the supplementation of whey and maltodextrin to which the athletes were already accustomed, but stipulated at the beginning of the study that the participants must not have used beta-alanine for at least six months before the beginning of the studies, and creatine for at least three months. However, no dietary controls were implemented.

Table 1. Characteristics of the studies evaluated.

Study	Participants	Exercise performed	Intervention (Supplementation)	Interval (days)	Result
Chung[6]	41 elite male and female swimmers	100m and 200m freestyle	4,8g for the first 28 days and 3.2g for the last 42 days	70	Improved race time in the first 28 days of supplementation and reduced blood lactate in the last 42 days
Mero[10]	13 male swimmers	2 sprints of 100m freestyle	4,8g per day	28	No change in performance
Painelli[11]	16 male and female swimmers	100m and 200m freestyle	3,2g for the first 7 days and 6.4g for the last 28 days	35	Improvement in race time (100m e 200m)
Brisola[12]	22 male swimmers	Sprint1 + 30min Swim + Sprint2	4,8g for the first 10 days and 6.4g for the last 18 days	28	Sprint1 time improvement
Brisola[13]	22 male swimmers	Tied swim (30s) + Free swim 200m + Barre Jump (30s)	4,8g for the first 10 days and 6.4 for the last 18 days	28	Improved strength in tied swimming (30s) and 200m freestyle swimming
Claus[14]	15 young male water polo players	RSA Test (Sprint and Throw) + Tied swim (30s) alternated with jump + Free swim 200m	6,4g per day	42	Improved time and throwing power in the RSA test; Improved strength in the jump; Improved performance in the 200m freestyle swim
Brisola[16]	22 male swimmers	Gradual effort in Tied swim + Total effort in Tied swim (3min)	4,8g for the first 10 days and 6.4 for the last 18 days	28	Improvement in strength associated with Vo2 max consumption
Norberto[15]	13 male and female swimmers	400m freestyle + Tied swim session (30s)	4,8g per day	42	No change in performance

Legend: NRSA: training in the ability of repeated sprints; VO2 max: maximum volume of oxygen.
Source: The authors (2023).

The study by Norberto[15] recruited participants who were not chronic users of nutritional supplements or anti-inflammatory medications. They received instructions not to consume alcohol, caffeine, and energy drinks for at least 12 hours before each measurement. Mero[10] did not stipulate any control over the participants' eating habits, controlling only supplementation and allowing the use of protein and carbohydrate based supplements, while prohibiting creatine and caffeine.

Differently, Chung[6] chose not to control the diet of the participants, only controlling for creatine, caffeine and sodium bicarbonate supplementation during the study. Meanwhile, Painelli[11] did not measure any diet and supplementation parameters of the participants.

The studies used different types of exercises and tests based on swimming and water polo training: (a) Chung[6] and Painelli[11] used the 100- and 200-meter freestyle, (b) Mero[10] used only two 100-meter freestyle sprints, (c) Claus[14] followed a water polo practice simulation training protocol consisting of eight 50-meter sprints followed by a maximum sprint from the edge to the middle of the pool where the athlete shoots the ball into the goal, thus determining time and strength parameters, (d) Norberto[15] analyzed 400-meter freestyle sessions followed by a 30-second tethered swim, observing time and strength patterns, while (e) Brisola and colleagues[12,13,16] performed similar exercises in their three studies, changing only the frequency, time performed and order of exercise, such that in Brisola[13] two repeated sprints interspersed by 30-minute swims were performed; Brisola[12] used a 30-second tethered swim followed by a 200-meter free swim and repeated jumps on the bar (in the pool) in a 30-second interval, and Brisola[16] used a gradual effort tethered swim for a period of 3 minutes.

Some of the studies were divided into competition phases. In Chung[6] the tests were conducted between two competitions (national and international) over a 10-week supplementation interval. The studies conducted by Brisola[12,13,16] started in the general preparatory phase of the season and ended in the competitive phase of each year. In contrast, the study conducted by Claus[14] lasted for six weeks, which were divided into a general preparation phase, specific training and pre-competition training during the last two weeks of training. Only one of the studies, by Norberto,[15] did not specify the period in which the protocol was performed.

Participants in five[6,11-13,16] of the eight studies received differing doses during the beta-alanine supplementation period, such that most received higher dosages in the initial days to increase intramuscular carnosine loading (saturation). In the remaining three studies, by Mero[10], Claus[14] and Norberto[15], the supplementation was administered in a uniform manner, without any increases or decreases in dosage over time.

In the protocol conducted by Chung,[6] a 4.8g/day dose was administered during the first 28 days and a maintenance dose of 3.2g/day was provided during the last 42 days. In the protocol used by Painelli,[11] a lower dose was administered during the first seven days (3.2g/day) and a higher dose during the last 28 days (6.4g/day). All three studies conducted by Brisola[12-13,16] followed the same supplementation protocol: a starting dose of 4.8g/day during the first 10 days and a final dose of 6.4g/day during the last 18 days.

The studies conducted by Mero[10] and Norberto[15] used a dosage of 4.8g/day throughout the supplementation period, while the study by Claus[14] used 6.4g/day.

The supplementation of 4.8g/day used by Mero[10] and Norberto[15] showed no effect on the performance of the participants, even using different training protocols and different study times. Claus[14] observed improvements in time and strength of participants in the RSA test (sprint and ball throw), 30-second tethered swim alternated with a bar jump and 200m freestyle swim.

Chung[6] observed an improvement in race times during the first 28 days of supplementation (beta-alanine saturation phase) and a reduction in blood lactate during the last 42 days (maintenance phase) (Figure 1).

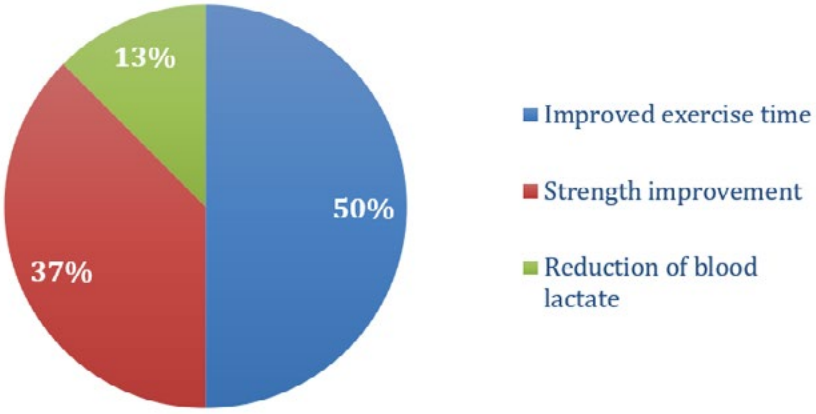

Figure 1. Results of the performance changes in the studies
Source: The authors (2023)

Discussion

Beta-alanine and the potential ergogenic effects of chronic supplementation in high-intensity exercises have become a topic of great interest within the sports community,[17] stimulating several studies that seek to measure the effectiveness of such supplementation in various high-performance sports. However, many contradictions can be found in the findings of these studies, which should be reviewed and analyzed in depth, specifically within each sport modality. Research in aquatic sports still shows contradictions and a lack of evidence of positive effects, when compared with other sports that stimulate physical exertion at the same levels as swimming and water polo.

In contrast to swimming and water polo, cycling is the modality that most stands out in studies and findings on this topic. Apparently, the long 10km time trial races that last more than 1 hour are not influenced by β-alanine supplementation, which can be explained by the fact that the predominant glycolytic pathway is aerobic.[7,18-19] However, in long-duration cycling races, high-intensity shots occur during attempts to overtake other cyclists, which increases the use of the anaerobic pathway and, consequently, muscle acidosis. At this point, when sprints of an average of 30 seconds were performed in this type of test, Van Thienen[19] showed that β-alanine supplementation can improve performance in sprints.

In the case of this study, analysis of the use of supplementation in water sports found that the average age of the 164 participants analyzed was 19 years, which can be explained by the fact that this age is associated with a higher level of intramuscular carnosine concentration, since this dipeptide can become depleted in older people.[2]

Analysis of the results showed that 50% of the studies found improvements in exercise time after receiving beta-alanine supplementation, while 37% reported gains in strength and 13% reported reduced blood lactate. The findings of the main studies on β-alanine supplementation on swimming performance are still divergent and even contradictory, although some studies indicate that this strategy is very likely to be effective in improving performance in 100m and 200m races, since these distances require a high production of lactate by the anaerobic pathway.[9]

Taking into account the parameters used in the studies, the training methods, time and intensity control, a strong impact can be observed on performance in swimming competitions in 100m and 200m races, since, similarly to creatine, beta-alanine does not promote weight gain, but allows for an increase in high intensity efforts.[20-21]

When conducting their study, Painelli and colleagues[11] found an improvement in swimming times in the 100m and 200m freestyle (1.4-2.1% improvement), as did Chung.[6] However, Chung,[6] even with a longer period of supplementation, was unable to find conclusive evidence of any improvement in performance after the fourth week of beta-alanine administration, observing only a decrease in lactate during the last 6 weeks, which had no ergogenic impact on the athletes.

Another factor that was also found not to contribute to changes in performance were doses lower than 4.8g in a short period of beta-alanine supplementation. This lack of change in performance is explained by Harris,[22] who demonstrates that achieving significant increases in intramuscular carnosine requires supplementation over a period longer than a minimum of four weeks with doses of 6.4g of beta-alanine, in order to promote increases of up to 64% in intramuscular carnosine content. The same conclusion is reached by Mero and colleagues[10] who showed that their study should have included higher doses of beta-alanine over a longer period of time in order to observe expected positive changes in performance, reaching the same conclusions as Baguet[2], who also emphasizes the need for a longer period of supplementation with higher doses, in order to achieve greater increases in intramuscular carnosine.

The results observed included evidence of a heightened ergogenic effect in studies that involved the administration of higher doses of beta-alanine, either in terms of duration or quantity, until the end of the protocol. Although no measurements of intramuscular carnosine levels were taken, the studies have as a general parameter that beta-alanine supplementation promotes an increase of approximately 50% in carnosine levels, which is confirmed by the study of Hill,[23] in which the administration of 4 to 6.4g/day of beta-alanine was shown to increase muscle carnosine concentrations by 59% after 4 weeks and 80% after 10 weeks. In their study, Chung and colleagues[6] discuss the dosages used, which were based on studies by Stellingwerff and by Harris that demonstrated the viability of performance enhancement with beta-alanine administration to increase muscle carnosine stores.[1,22]

However, six of the eight studies presented unclear results (probable beneficial effect at competitive levels) in which improvements in strength and time were small, but significant. This may have an ergogenic effect at a competitive level, where a few seconds and a stronger throw can have positive effects on the final result in a competition, as argued by Claus.[14]

Another aspect of the use of beta-alanine is its use as a supplement in conjunction with sodium bicarbonate. According to a study by Mero[10] the purpose of this association would be enhanced supplementation when both substances are used together. However, no relevant results have been observed from this association.

One of the limiting factors in the studies was metabolic adaptive interference arising from training and competitions during the protocols used. These activities can reduce blood lactate, and consequently improve the parameters used to identify the ergogenic effect of beta-alanine, as cited by Norberto.[15] Another limiting factor observed among all the studies was that the samples of participants are quite small for the purposes of analyzing performance in a competitive sport with various variables that determine ergogenic effects.

In addition, the studies analyzed were unable to measure intramuscular carnosine, a dipeptide that contributes to the buffering of hydrogen ions and acts to decrease muscle acidosis. This limiting factor occurs because measurement is not feasible, since it would require interventions such as biopsies for the measurement of intramuscular carnosine, which, according to Chung,[6] would be unrealistic to be performed on elite athletes while they were preparing for competition.

Given these limiting factors, one of the parameters used to find performance improvement was the decrease and control of lactate, as exemplified by Kontic,[24] since anaerobic lactate endurance is a significant predictor of offensive and defensive agility for water polo, as confirmed by almost all authors. The exception is Chung and colleagues[18] who did not describe the improvement in blood lactate as having a stronger relationship with training-generated adaptions than simple beta-alanine supplementation, despite finding a reduction of the organic compound in their results. On the other hand, other studies that found reductions in lactate showed that this decrease was due to adaptations generated by training and not by the supplementation itself.

Conclusion

The results presented in this study confirm a possible beneficial effect of beta-alanine on the performance of swimmers and water polo players, through a decrease in muscle acidosis and, consequently, fatigue. When associated with physical exercise and not used in isolation; however, the protocol applied has significant influence on the results obtained.

The best beta-alanine supplementation protocols analyzed consisted of the administration of doses greater than or equal to 4.8g per day for a least a 4-week period, since these studies used doses of up to 6.4g per day without an initial saturation period. Only one study did not show improved performance with this protocol pattern.

One can conclude that the improved performance associated with the use of beta-alanine is presented in such parameters of aquatic sports as time in the 100m and 200m freestyle, sprints, and throwing force. The supplementation of beta-alanine should occur every day, regardless of the time of day it is administered. However, in order to avoid the side effect of paresthesia, the daily dose should be split into smaller dosages administered at 2-hour intervals.

For better results, in future studies, we suggest that the intramuscular carnosine of the swimmers and athletes be measured in conjunction with the supplementation of beta-alanine during the protocol used.

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Invasive disease by *Streptococcus pneumoniae*: a case report and a discussion about the immunization rates in older adults in Brazil

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Abstract

Invasive disease by *Streptococcus pneumoniae* has a high mortality rate, especially in older adults and children. This paper presents the progression and prognosis of a case of invasive vaccine serotype pneumococcal disease in an elderly woman and promotes a discussion about the monitoring of the pneumococcal vaccine rate among older adults in Brazil.

Keywords: *Streptococcus pneumoniae*; Invasive disease; Vaccine; Pneumococcal vaccine.

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BJHBS, Rio de Janeiro, 2023;22(2):126-129
DOI: 10.12957/bjhbs.2023.77287
Received on 18/07/2023. Approved on 27/08/2023.

Introduction

Streptococcus pneumoniae is a gram-positive coccus that belongs to the normal microbiota of the upper respiratory tract.[1] This pathogen is associated with invasive infections, such as pneumonia, meningitis, sinusitis, endocarditis and bacteremia that affect all ages and genders. These infections present high mortality rates and their evolution is usually acute and aggressive, even with correct antimicrobial therapy.[1-5]

The Advisory Committee on Immunization Practices (ACIP) recommends pneumococcal vaccines for the prevention of pneumococcal diseases. Three vaccines are widely distributed: 10-valent pneumococcal conjugate vaccine (PCV10), 13-valent pneumococcal conjugate vaccine (PCV13) and 23-valent pneumococcal polysaccharide vaccine (PPSV23).[6] Although some of these vaccines are widely available through the public health system, pneumococcal diseases represent an important public health and economic issue in Brazil.[7]

The prevalence of pneumococcal serotypes changes over time and according to stages of economic development. Due to Brazil's continental dimensions, the prevalence of pneumococcal serotypes differs among regions and states. Brazilian studies note that invasive pneumococcal diseases decreased after the implementation of PCV10 in the routine immunization program, but colonization and infections promoted by non-PCV10 serotypes, as such as 3, 6A, 6C, and 19A, persist as an important health public issue.[4,7-9]

Although S. pneumoniae is responsible for many invasive diseases,[2-5] as shown in the literature, regular investigation and monitoring of the pneumococcal serotypes involved in aggressive diseases is rare in Brazilian hospitals. In addition, most epidemiological studies focus on the young population,[8-9] with few data on older adults. This paper reports an invasive disease by *S. pneumoniae* vaccine serotype in a woman (Bioethics Committee/CAAE: 01247512.3.0000.5259).

Case report

A 64-year-old woman was admitted to an emergency room complaining of intense pain in the cervical region, as well as headaches and dysarthria with progressive mental confusion during the previous week. The patient's medical history showed dyslipidemia, Wolff-Parkinson-White syndrome, high systemic blood pressure and splenectomy in childhood.

During the initial clinical exams, the patient presented mental confusion, low arterial pressure and signs that suggested an inflammatory process in the meninges (nape hardness, positive Kernig's sign, and positive Brudzinski's sign).

The patient underwent a computer tomography of the head (CT scan). The blood cell count showed a leukemoid reaction, 36,000 leukocytes with 83% neutrophils and 8% segmented. A lumbar puncture found xanthochromia and hemorrhagic cerebrospinal fluid, with 20cm H_2O pressure; 213mg/dL of total proteins; 20mg/dL of glucose; 192mm leukocytes^3; 75% of neutrophils; and 90 mg/dL of lactate.

After admission to the intensive care unit (ICU), the patient stayed in an isolation room and entered sepsis protocol during the first hour. The infectious diseases physician recommended antimicrobial therapy to cover pulmonary and meningeal focuses until the etiologic agent was identified with certainty.

A transthoracic echocardiogram was performed, which showed normal systolic function and diastolic dysfunction with a restricted pattern in the left ventricle and normal function in the right ventricle. It also showed a heterogeneous mobile lesion adherent image, suggesting vegetation with a possible abscess in the mitral valve, moderate tricuspid valve regurgitation, moderate to severe mitral valve regurgitation, and a normal pericardium.

Two of three blood cultures yielded Gram-positive cocci, alpha-hemolytic when cultured on blood agar, with optochin sensitivity. MALDI-TOF (matrix-assisted laser desorption/ionization-time of flight) analysis identified the microorganism as *S. pneumoniae*, with a score higher than 2.0.

The antimicrobial susceptibility test revealed susceptibility to vancomycin, clarithromycin, and ceftriaxone, resulting in the discontinuation of the administration of vancomycin and clarithromycin and the maintenance of the ceftriaxone treatment.

After one day of medical care in the ICU, the patient evolved to acute respiratory failure, prostration, depression in consciousness levels and acute renal failure. The team performed orotracheal intubation, while immediately starting renal dialysis and amine-vasoactive therapy.

After two days, her hemodynamic status aggravated, with 17.6mg/dL of lactate. The patient evolved to disseminated intravascular coagulation, with multiple organ failure, septic shock, and cardiac shock, and died six days after hospitalization.

The PCR protocol,[10] used to identify the capsular polysaccharide, classified the strain as 22F serotype. The strain was stored at -80°C in Tryptic Soy Broth (TSB) (Difco, USA) with 20% glycerol in the Microbiology and Immunology Department of the State University of Rio de Janeiro.

Discussion and conclusions

Streptococcus pneumoniae is a frequent cause of bacterial pneumonia among people of all ages and an important etiological agent of invasive infections, including meningitis and endocarditis.[1] Mortality from pneumococcal infection is at its highest level since the development of penicillin in the early 1940s.[11] Each year, approximately 14.5 million episodes and 500,000 deaths by invasive pneumococcal disease (IPD) occur in children.[12]

Fever is considered a frequent clinical manifestation in pneumococcal endocarditis, and even if both cardiac valves happen to be involved, damage to the aortic valve is the most prevalent.[2] The patient in question did not present fever and the transthoracic echocardiogram showed mitral valve involvement, aspects not commonly found in the literature.

The triad pneumococcal pneumonia, pneumococcal meningitis and pneumococcal endocarditis (Osler's triad), known as the Austrian Syndrome, is associated with a high mortality rate.[5] The patient presented no clinical symptoms suggestive of bacterial pneumonia. However, no investigatory exams were performed, so the syndrome remained undiagnosed. More detailed investigation of the presence of Osler's triad should be performed in patients with invasive pneumococcal infections.

Correct antimicrobial treatment is crucial in the first hours following a suspected pneumococcal infection. According to the European Society of Cardiology guidelines, infective endocarditis due to penicillin-susceptible S. pneumoniae requires short-term (2-week) treatment that combines penicillin with gentamicin. Guidelines recommend high doses of cephalosporins or vancomycin for penicillin-intermediate or -tolerant strains without meningitis. Cases with meningitis should be treated with ceftriaxone or cefotaxime alone or in association with vancomycin according to the antibiotic susceptibility pattern.[13] The invasive strain reported was susceptible to all antimicrobial agents usually used for the treatment of pneumococcal infections. Since the exams suggested the presence of meningitis, treatment with ceftriaxone was continued.

The implementation of pneumococcal vaccines in the public health system and the widespread immunization of the population are regarded as effective practices for preventing pneumococcal invasive disease.[6,7] In the Brazilian public health system, the national vaccination protocol includes PCV10.[14] PCV13 is available in private clinics[9] and for cancer and immunocompromised patients, while PPSV23 is suitable for patients with chronic disease, older adults, and indigenous peoples.[14] Although PPSV23 presents purified polysaccharide antigen for 23 serotypes, including serotype 22F, the patient was not tested for immunization.

Increases in life expectancy, the appearance of antibiotic-resistance bacteria and the need to prevent aggressive diseases suggest that pneumococcal immunization in older adults remains a significant public health issue.[15] In Brazil, most papers investigating pneumococcal colonization and their microbiological profile focus on the younger strata of the population.[4,8-9] Few studies report on immunization rates and the prevalent serotypes involved in pneumococcal diseases in older adults, which reveals a gap in the literature. The future development of vaccines and the prevention of IPD in Brazil require further research about the monitoring

and distribution of serotypes associated with invasive infections and the PPSV23 immunization rate in older adults.

Infective endocarditis due to *Streptococcus pneumoniae* is associated with a high mortality rate. This case report shows the poor prognosis and the importance of monitoring pneumococcal disease in Brazil, especially in older adults, as well as emphasizes the importance of monitoring the PPSV23 immunization rate in older adults to prevent invasive pneumococcal infections.

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BJHBS

The challenge of carbapenem resistance

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The global resistance of microorganisms to antimicrobials is one of the most important public health challenges in recent decades. This problem has gained even more relevance in the case of Gram-negative microorganisms, resulting in a shortage of medicines for the treatment of infections caused by these multi-resistant bacterial agents.

Charting the evolution of this resistance among microorganisms in the hospital environment is essential for: the projection of resistance in the coming decades; the rational use of antimicrobials; and the adoption of measures to minimize the impact of the resistance in this scenario.

The most modern antimicrobial agents, which act on multi-resistant bacteria, are expensive, which limits their availability and makes their use even more difficult, especially in the public health networks of developing countries. Moreover, new drugs for treatment of multidrug-resistant infections are often unavailable due to their high cost.

In recent years, the Pedro Ernesto University Hospital (HUPE) achieved recognition in the health care sector of the State of Rio de Janeiro because of its quaternary care and by offering a range of specialties that serve patients who require lengthy hospital stays and often need invasive devices, which favor colonization/infection by hospital microorganism that are generally resistant to three or more classes of antimicrobials. Therefore, it is necessary to use these anti-infectious agents, which act on multi-resistant bacteria, to treat infections developed during hospitalization. Furthermore, the hospital also treats patients transferred from other institutions, who may bring multi-resistant microorganisms with them, which makes antimicrobial therapy more complex and challenging.

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BJHBS, Rio de Janeiro, 2023;22(2):130-132
DOI: 10.12957/bjhbs.2023.80042
Received on 24/10/2023.

Resistance to antimicrobials in Gram-negative microorganisms that colonize or infect patients occurs mainly through the production of enzymes encoded by genes that can be located on plasmids and can be transferred to other microorganisms.

Extended-spectrum beta-lactamases (ESBL) were found in the 1980s to cause resistance in third and fourth generation cephalosporins.[1] This fact led to a more frequent use of carbapenems, which are stable to these enzymes. At the end of the 1990s,[2] resistance to carbapenems was detected due to the production of enzyme encodes by the bla_{KPC} gene. Other genes, such as bla_{IMP}, bla_{VIH}, and bla_{NDM} were detected at a later date, between 2001 and 2005.

The article "Prevalence of carbapenem-resistant *Enterobacteriaceae* in and out-of-hospital environments", by Olivella and colleagues,[3] in which 30 patients hospitalized at HUPE-

UERJ were evaluated, identified 13 (43.3%) carbapenem-resistant strains. One third patients from the community were analyzed, in 13 (29%) of which, carbapenem-resistant strains were identified. According to Nordmann,[4] "it is possible that, and of great concern for carbapenem resistance, a process similar to that for ESBL-producing strains decades ago will occur, causing endemic infections in the community".

Given this scenario, we sought to evaluate the occurrence of these resistances in Gram-negative microorganisms collected from HUPE patients during two periods: from 2005 to 2006 and 2022.[5]

During the first period, the presence of beta-lactam resistance genes was investigated in 44 microorganisms isolated from various clinical materials, and Polymerase Chain Reaction (PCR) tests and phenotypic tests were conducted for sensitivity to antimicrobials, including carbapenems. PCR is capable of detecting *bla*$_{SHV}$, *bla*$_{TEM}$, and *bla*$_{CTXM}$ genes. The phenotypic tests carried out did not detect resistance to carbapenems in these strains. During this period, 28 strains of *Klebsiella pneumoniae* were identified and, in these microorganisms, *bla*$_{CTXM}$ were detected in 12 patients (42.8%). At the same time, 6 *Escherichia coli* strains were detected, in which 3 (50%) occurrences of *bla*$_{CTXM}$ were found. No resistance genes were detected in the 10 other microorganisms isolated during this period. In phenotypic tests, no carbapenem resistance was detected.

Approximately 16 years later, samples isolated from blood cultures of 90 patients, using a multiplex PCR (Biofire FilmArray BCID) capable of detecting the *bla*$_{CTXM}$, *bla*$_{IMP,}$ *bla*$_{VIM}$, *bla*$_{NDM}$, *bla*$_{KPC}$ and *bla*$_{OXA48}$ genes, identified 29 *K. pneumoniae* strains and 23 *E. coli* strains, in addition to 38 other Gram-negative microorganisms. The most frequent genes were *bla*$_{CTXM}$ (which was detected in 18 (48.65%) cases); *bla*$_{KPC}$, detected in 12 (32.43%) cases; *bla*$_{NDM}$ in 5 (13.51%); and *bla*$_{OXA48}$ in 2 (5.40%).

Therefore, high proportions of the *bla*$_{CTXM}$ genes were detected in both periods, although with greater frequency in 2022, despite the decrease in the prescription of third generation cephalosporins at HUPE-UERJ in recent years.

The presence of genes encoding resistance to carbapenems was observed in 2022, with a high frequency (51.34%) of Gram-negative strains resistant to antimicrobial beta-lactams, which is extremely worrying due to the potential for spread of this resistance.

The high frequency of *K. pneumoniae* in 2022 is worrying since this agent "is a notorious collector of multidrug resistance plasmids". The rapid global dissemination of KPC producing *K. pneumoniae* implies multiple forms of transmission.[6]

The fact that the *bla*$_{CTXM}$ genes did not decrease during the most recent period and that they are often associated with carbapenem resistance genes shows a difference from the case of oxacillin-resistant *Staphylococcus aureus*, where the I, II, III genetic cassettes were replaced by IV, V, VI (CA-MARSA) ones.[7]

The transfer of antimicrobial resistance genes is a challenge, and the containment of resistance is more complicated in hospitals with old architecture, with wards that contain a large number of beds. These characteristics indirectly increase hospitalization costs and morbidity/mortality numbers and can cause a gradual increase in difficulties in controlling bacterial resistance for the health network in general.

The complexity of the phenomenon of microbial resistance to antimicrobials causes difficulties in addressing hospital infections, and the spread of resistance genes is likely to intensify in the coming years. A systemic approach, including investment in research and a reduction in the length of hospital stays, is necessary to reduce bacterial resistance, which is a serious global problem.

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Brazilian Journal of Health and Biomedical Sciences
Paper submission

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Copyright/conflicts of interest agreement: after the final approval, authors must send the copyright transfer agreement signed by the first author representing each additional author. In this agreement, it must be stated any conflicts of interest.

Introduction letter: a letter that must come with the submitted paper and contains at least the following information:

A statement that the paper has not been submitted for publication in another journal;

Recommendation of two reviewers (specialists) for consulting in the scientific field of the submitted paper + e-mail, preferably who are not from the same institution as the authors. The Editorial Board may or may not choose any of these consultants;

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If the authors had assistance from technical writers or language reviewers, it must be explicitly stated in the introduction letter, along with the assurance that the authors are fully responsible for the scientific content of the paper.

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Types of papers

1. Original papers: Papers resulting of original research. Maximum of 5,000 words (excluding abstract and references) and five images or tables. Maximum of 40 listed references. They must be submitted in the following format:

Abstract: must be written in English with a maximum of 250 words. Must follow the structured abstract model, with mandatory introduction, objective(s), methodology and resources, results and discussion, conclusion(s). It is well known that the abstract gets more visibility and distribution than the full text of the paper. Therefore, it must contain the essential information in the paper, but cannot be just a patchwork of sentences from it. It must be succinct and direct, highlighting what is most important in the full text in order to encourage a full reading. In the conclusion, all results must be related to the objectives of the study. The discussion must assert the contribution of the results to the body of knowledge about the subject of research.

Keywords: three to six terms related to the subject must be given, separated by semicolons, according to MeSh (Medical Subjects Headings) for English.

Full text

Introduction: it must be short and present the purpose (context and justification) of the study, including a short review of relevant studies about the subject, mentioning any recent progress, and referencing just what is appropriate.

Methodology and resources: this section must briefly present all the information needed for other researchers to replicate the study. Adopted procedures must be clearly described, as must the analyzed variables and tested hypotheses. Definitions must be given whenever necessary. Population, sample, and measurement instruments must be described and information about data gathering and processing must be given. If possible, validity scores must be included. Methods and techniques used must be duly detailed, including statistic methods. New or substantially modified methods must be described, with a justification for its use and mention of its limitations. Research ethics must be observed. Authors must explicitly state that the research was done within ethical standards and with the approval of an ethics committee.

Results: this section must be a concise report of all new information found, with minimum personal bias and judgment. The data must be presented in a logical sequence, starting with the most important information. Data from tables and images must not be repeated, but briefly referred to. It must state the significance of the new data and the relevance of the new findings in relation to established theories and to scientific literature. In this section must also be mentioned the limitations of the present work, as well as its implications for future research. Finally, conclusions must be included in this section, always related to the initially stated objectives.

Acknowledgments: must be concise and limited to people and institutions that contributed to the research in some degree, but could not be included as authors.

In-text citations: BJHBS follows the Vancouver style, according to the general rules of The NLM Style Guide for Authors, Editors, and Publishers, second edition (www.ncbi.nlm.nih.gov/books/NBK7256/). For in-text citations, use Arabic numerals superscript, 1 without spaces, right after a word or punctuation: "Parkinson's Disease[1] description began in the 1950s,2 when..." In some cases, the names of the authors may figure in the text: "Phillips[12] analyzed several conditions of..."; and up to two authors can be named: "Handel and Matias[15] conducted a study about..." However, when the number of authors is three or more, the first author must be named along with the expression "and colleagues.": "Silveira and cols.[13] have proposed a new methodology..."

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Tables and/or images: up to a maximum of five, including the authorship and/or source.

Tables: must be created in dedicated software, such as Excel. The width must be proportional to one page in the current layout. The font must be Arial, size 9, single space. Tables must be imported to and submitted in a text file: .doc/.docx (Microsoft Word), .rtf (Rich Text Format), or .odt (Open Document Text). They must be assigned a number in ascending order and receive a title and/or subtitle explanation. They must also be referenced within the text. The content of a table must not replicate that of an image nor vice versa. Their numbers must be assigned according to

the order in which they are referenced in-text. All abbreviations must be explained with a legend below the table. There must be the source from which the table was extracted and/or the authorship of it, this information must be written below the table, after the legend for the abbreviations, if any.

Images: can be photos, illustrations, graphics, drawings, etc. Images must be submitted as separate files (.tiff or .jpeg). They must be assigned a number in ascending order and receive a title and/or subtitle explanation. They must also be referenced within the text. All abbreviations must be explained with a legend below the image. There must be the source from which the image was extracted and/or the authorship of it, this information must be written below the image, after the legend for the abbreviations, if any.

2. Clinical cases:

Case report: usually it describes one to three patients or a family case. The text must be up to 2,000 words long, with up to three tables or images and up to 25 references. The abstract must be no more than 100 words long.

Clinical case solution: it must contain a step- by- step description of the decision process of clinical cases. Patient information must be presented to one or more clinical experts in stages (text in bold) to simulate the way information is made available in clinical practice. The expert must answer (text in regular font) as new information is added, sharing their reasoning/arguments with the reader. The text must be up to 2,500 words long, and must have up to 15 references.

3. Literature review:

It must be about subjects relevant to medical practice. These will form a section about the common theme of each issue. These are limited to 5,000 words (excluding abstract and references) and a maximum of five images and/or tables. Maximum of 40 listed references. Literature reviews will be submitted for the editorial board analysis under invitation by the guest editor of this section, and must conform to the following standards:

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keywords: three to six terms related to the subject must be given according to MeSh (Medical Subjects Headings). Keywords must be separated by semicolons.

Literature reviews may fall into two types:

a. Systematic review and meta-analysis - Through a synthesis of original studies' results, the paper must answer specific relevant health sciences questions about the theme of its issue (see BJBHS's focus). It must detail the search process to find the original studies, selection criteria, and synthesis procedures for the results of the reviewed studies (which may or may not be meta-analysis procedures).

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Acknowledgments: must be concise and limited to people and institutions that contributed to the research in some degree, but could not be included as authors.

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