CAMILLA ASTLEY AMARAL PEDROSO

Efeito do treinamento físico sobre a qualidade de vida relacionada à saúde em crianças e adolescentes após a COVID-19 e síndrome inflamatória multissistêmica pediátrica

(Versão corrigida. Resolução CoPGr n° 6018, de 13 de outubro de 2011. A versão original está disponível na Biblioteca FMUSP).

São Paulo

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Tese apresentada à Faculdade de Medicina da Universidade de São Paulo para obtenção do título de Doutor em Ciências

Programa de Ciências do Sistema Musculoesquelético

Orientador: Prof. Dr. Bruno Gualano

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"To attempt to reach above and beyond expectations, no matter the circumstance."

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SUMMARY

RESUMO

Pedroso CAA. Efeito do treinamento físico sobre a qualidade de vida relacionada à saúde em crianças e adolescentes após a COVID-19 e síndrome inflamatória multissistêmica pediátrica [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2025.

Introdução: A COVID-19, apesar das baixas taxas de infecção na população pediátrica, pode levar a casos graves, especialmente em crianças com doenças crônicas, resultando em complicações e órgãos e sistemas como inflamação persistente e hospitalizações prolongadas. A síndrome inflamatória multissistêmica pediátrica (SIM-P), é condição grave associada à COVID-19. Ambas as condições podem levar a disfunções multissistêmicas e afetar negativamente a qualidade de vida relacionada à saúde (HQROL do inglês: *Health-related Quality of Life*), ocasionando comprometimentos físicos, cardiorrespiratórios e psicossociais. Neste cenário, o exercício físico surge como uma intervenção promissora. O objetivo desta tese foi investigar os impactos da COVID-19 e da SIM-P na capacidade física e cardiorrespiratória de crianças e adolescentes, bem como avaliar os efeitos de um programa de treinamento físico domiciliar (HBET do inglês: *Home-based exercise training*) sobre a HRQOL e outros parâmetros fisiológicos por meio de ensaio clínico randomizado (Clinicalrials: NCT04659486). **Métodos e resultados:** Esta tese foi composta pela compilação de quatro artigos publicados. No primeiro artigo, utilizamos a técnica 13 N PET-CT (do inglês: *Positron Emission Tomography with 13N-Ammonia*) padrão-ouro para avaliar perfusão miocárdica e reserva de fluxo coronariano (CFR, do inglês *Coronary Flow Reserve*). Além disso, avaliamos a dilatação mediada por fluxo braquial (b-FMD, do inglês *Brachial Flow-Mediated Dilation*), aptidão cardiorrespiratória pelo (CPET, do inglês *Cardiopulmonary Exercise Test) e* as manifestações clínicas durante a fase aguda da doença. Nesta série de casos, observou-se comprometimentos significativos, como isquemia miocárdica, disfunção endotelial e redução na capacidade cardiorrespiratória, destacando a gravidade das sequelas cardiovasculares pós-MIS-C. No segundo artigo, avaliamos crianças e adolescentes, em sua maioria imunocomprometidos, de 3-6 meses após a COVID-19, comparando-os a um grupo controle pareado por sexo, idade, índice de massa corporal e presença de doenças crônicas prévias. Os resultados mostraram redução na capacidade cardiorrespiratória, com menor limiar anaeróbico (VO_2VAT, do inglês *VO_2 at Ventilatory Anaerobic Threshold)* e tempo total de exercício, além de disfunção endotelial,

identificada por valores reduzidos de b-FMD, quando comparados a controles não infectados. A severidade da doença, síndrome pós-COVID-19 e hospitalização não foram associadas à menor capacidade cardiorrespiratória ou disfunção endotelial. O terceiro artigo, foi a continuação da série de casos. Incluímos seis pacientes com idade entre 7 e 16 anos, sendo cinco previamente saudáveis e um com diagnóstico de diabetes melitus tipo I. Três pacientes completaram a intervenção, enquanto os outros três foram utilizados como grupo controle em uma amostra de conveniência. Os pacientes exercitados apresentaram uma melhora da HRQOL, da CFR, avaliada pelo 13 N PET-CT, na função cardíaca por ecocardiografia e na capacidade cardiorrespiratória. Em contrapartida, os que optaram pela não deram início ao protocolo HBET tiveram uma recuperação mais lenta, com a piora de alguns desfechos clínicos quando comparados ao seu estado basal. Por fim, o quarto artigo foi um ensaio clínico randomizado que avaliou a mesma intervenção sobre a HRQOL e em parâmetros cardiovasculares e metabólicos em pacientes pediátricos pós-COVID-19. Foram randomizados 32 pacientes para os grupos HBET ou (CTRL, do inglês control). Os resultados mostraram tendência de melhora no VO_2 VAT e melhorias significativas na frequência cardíaca de pico, recuperação cardíaca em 1 minuto e resposta cronotrópica no grupo HBET. Não foram observadas diferenças na HRQOL e outros parâmetros, como função endotelial e ecocardiografia. **Conclusão:** Nossos resultados destacam a importância de avaliar sistematicamente crianças e adolescentes acometidos pela COVID-19, monitorando ao longo do tempo as possíveis disfunções sistêmicas e funcionais, especialmente em pacientes imunocomprometidos, nos quais a recuperação da capacidade física e da função endotelial parece ser comprometida, embora os mecanismos ainda sejam desconhecidos. Além disso, destacamos a importância de implementar programas estruturados de exercício físico para auxiliar na reabilitação funcional e cardiovascular, tanto para infecções mais leves quanto para aquelas que exigiram hospitalização. Por fim, estudos multidisciplinares e randomizados devem ser conduzidos para confirmar esses achados preliminares e aprofundar a compreensão dos efeitos da COVID-19 nessa população vulnerável.

Palavras-chave: Pediatria. COVID-19. Síndrome inflamatória multissistêmica pediátrica. Exercício físico. Capacidade cardiorrespiratória. Qualidade de vida.

ABSTRACT

Pedroso CAA. Effects of exercise training on health-related quality of life in children and adolescents after COVID-19 and pediatric multisystem inflammatory syndrome [thesis]. São Paulo: “Faculdade de Medicina, Universidade de São Paulo”; 2025.

Introduction: Despite the low infection rates of COVID-19 in the pediatric population, severe cases can occur, particularly in children with pre-existing chronic conditions, leading to complications, persistent inflammation, and prolonged hospitalizations. Multisystemic Inflammatory Syndrome in Children (MIS-C) is a severe condition associated with COVID-19. Both conditions can result in multisystem dysfunctions, negatively impacting health-related quality of life (HRQOL) and causing physical, cardiorespiratory, and psychosocial impairments. In this context, physical exercise emerges as a promising intervention. The objective of this thesis was to investigate the impacts of COVID-19 and MIS-C on the physical and cardiorespiratory capacity of children and adolescents, as well as to evaluate the effects of a home-based exercise training program on HRQOL and other physiological parameters through a randomized controlled trial (*ClinicalTrials*: NCT04659486). **Methods and Results:** This thesis consists of four published articles. In the first article, we used the 13N PET-CT technique (*Positron Emission Tomography with 13N-Ammonia*), the gold standard for evaluating myocardial perfusion and coronary flow reserve (CFR). Additionally, we assessed brachial flow-mediated dilation (b-FMD), cardiorespiratory fitness through cardiopulmonary exercise testing (CPET), and clinical manifestations during the acute phase of the disease. This case series revealed significant impairments, such as myocardial ischemia, endothelial dysfunction, and reduced cardiorespiratory capacity, highlighting the severity of cardiovascular sequelae in this population. In the second article, we evaluated children and adolescents, most of whom were immunocompromised, with a history of COVID-19. Results were compared with a control group matched by sex, age, body mass index, and pre-existing chronic conditions. The findings demonstrated reduced cardiorespiratory capacity, with a lower ventilatory anaerobic threshold (VO_2 VAT) and total exercise time, in addition to endothelial dysfunction, as evidenced by decreased b-FMD values compared to non-infected controls. The third article was a case series including six patients aged 7 to 16 years, five

of whom were previously healthy, and one with type I diabetes mellitus. Three patients completed the intervention, while the other three served as a convenience control group. Patients in the exercise group showed improvements in HRQOL, CFR assessed by 13N PET-CT, cardiac function via echocardiography, and cardiorespiratory fitness. In contrast, those who discontinued the intervention exhibited a slower recovery, with worsening of some clinical outcomes compared to baseline. Finally, the fourth article was a randomized controlled trial evaluating the same intervention on HRQOL and cardiovascular and metabolic parameters in post-COVID-19 pediatric patients. A total of 32 patients were randomized into intervention (HBET, *Home-Based Exercise Training*) or control (CTRL) groups. The results showed a trend towards improvement in VO_2 VAT and significant improvements in peak heart rate, one-minute heart rate recovery (HRR1min), and chronotropic response in the HBET group. No significant differences were observed in HRQOL, endothelial function, or echocardiography parameters. **Conclusion:** Our findings highlight the importance of systematically evaluating children and adolescents affected by COVID-19, monitoring potential systemic and functional dysfunctions over time, particularly in immunocompromised patients whose recovery of physical capacity and endothelial function appears impaired, although the mechanisms remain unknown. Furthermore, our results reinforce the need to implement structured physical exercise programs to aid in functional and cardiovascular rehabilitation. Finally, multidisciplinary and randomized studies are needed to confirm these preliminary findings and further understand the effects of COVID-19 in this vulnerable population.

Keywords: Pediatrics. COVID-19. Multisystemic inflammatory syndrome in children. Exercise training. Cardiorespiratory fitness. Quality of life.

1 INTRODUCTION

1.1 COVID-19 and Multisystemic Inflammatory Syndrome in children and adolescents

The pandemic caused by the etiological agent known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and its associated disease, Coronavirus Disease 2019 (COVID-19), has become a major public health issue, with more than 7 million deaths recorded by January 2025 (1). Among confirmed cases, it is estimated that 15.6 million, representing 17.9% of the cumulative total, occurred in children and adolescents (2). Although pediatric patients have the same susceptibility to SARS-CoV-2 infection than adults, the disease generally presents with a milder course or even asymptomatic cases, with a recovery in 1–2 weeks after symptoms onset (3–5). For example, a multicenter study conducted in Europe revealed that 80% of infected children and adolescents had symptoms resolved without apparent sequelae (6).

The reasons related to the lower incidence of COVID-19 in children could be associated with the reduced expression of angiotensin-converting enzyme (ACE-2) receptors, which are involved in facilitating SARS-CoV-2 entry into cells (7–9). This reduced expression is particularly evident in the nasal epithelium, where children exhibit the lowest levels of ACE-2 compared to adults (10). Additionally, the lower expression of ACE-2 in bronchial epithelial cells further contributes to the decreased susceptibility of children to severe respiratory symptoms (11). These age-related differences in ACE-2 expression and the robust innate immune response may limit the virus's ability to invade and replicate. This also explains why children are more likely to show non-respiratory symptoms, fact that contributes to the milder clinical outcomes observed in this population (12).

On the other hand, children and adolescents are not exempt from developing severe forms of infection, with a subset experiencing significant complications that can lead to hospitalization and long-term health consequences. For instance, some comorbidities related to the cardiovascular and pulmonary system, obesity, and diabetes mellitus can alter the body's epigenetic programming, leading to increased expression of ACE-2, facilitating the process of viral entry into pulmonary and epithelial cells (13,14), predisposing these patients to experiencing more severe symptoms of

the disease. In adults, the presence of preexisting chronic conditions, including treatments with immunosuppressants that impair the immune system are significant factors associated with the severity of COVID-19 (15). Similarly, in children, comorbidities such as chronic heart disease, respiratory diseases, and obesity have also been linked to more severe cases of COVID-19. Recently, a study conducted in a tertiary hospital in Sao Paulo found that approximately 75% of pediatric patients who required mechanical ventilation during acute phase of COVID-19 had at least, one preexisting condition (16).

Another condition that has been well described in the literature was the multisystem inflammatory syndrome in children (MIS-C). This is a rare, but potentially severe disease that emerged during COVID-19 pandemic. MIS-C typically manifests weeks after SARS-CoV-2 infection and may be associated with severe cases of COVID-19, characterized by an abnormal immune response and hyperactivating body systems that leads to a high inflammatory state. The clinical picture of MIS-C includes fever, abdominal pain, diarrhea, vomiting, increased markers of inflammatory activity (i.e., C-reactive protein, erythrocyte sedimentation rate, ferritin, tumor necrosis factor and interleukin 6 and 1β), coagulation markers (i.e., prothrombin time, activated partial thromboplastin time, D-dimer), and alterations in the cardiac biomarkers (i.e., troponin, N-terminal pro B-type natriuretic peptide [NT-proBNP]) (17–21). The features are remarkably similar to Kawasaki disease, macrophage activation syndrome, and toxic shock syndrome (18–20).

In our institution, a study found that 6/66 (9%) of the pediatric COVID-19 patients were diagnosed with MIS-C, and they exhibited a more severe clinical presentation and higher mortality rate compared to those who had only COVID-19 (22). Stands out that some risk factors such as age, viral load, and comorbidities can be associated with the severity of the disease and possibly the onset of MIS-C (23,24). Furthermore, a multicenter study involving 16 hospitals in Sao Paulo state included 101 pediatric patients diagnosed with MIS-C (28). Among these patients, 73.3% required intensive care, 20.8% needed mechanical ventilation, and 35.6% required inotropic support (28). The mortality rate in this study was 3.9%, with three of the four deaths occurring in previously healthy children. It is important to note that other studies stated a mortality rate between 1.1% to 5% after MIS-C (25,26). Although these numbers are relatively low, they may still be considered significant when compared to other pediatric conditions.

The cardiovascular system plays an important role in the elevated mortality rates observed in pediatric patients diagnosed with MIS-C. For example, Valverde (2021) evaluated 286 children and adolescents with MIS-C, finding that 93% had myocardial involvement, 40% experienced shock, and 35% developed arrhythmias (27). These cardiovascular complications often demand advanced therapeutic interventions, including hemodynamic support, mechanical ventilation, and, in the most severe cases, extracorporeal membrane oxygenation (ECMO) (28,29).

The microcirculation and coronary flow reserve (CFR) are also significantly impacted in MIS-C (30–32), contributing to myocardial ischemia, impaired cardiac and endothelial function. These abnormalities underline the critical need for close monitoring and advanced diagnostic tools to assess the extent of these complications and manage these cardiovascular impairments effectively.

Overall, the multisystemic nature of this syndrome, with the cardiovascular system being particularly affected by the intense inflammatory response, contributes to severe clinical manifestations that can significantly impair and reduce health-related quality of life (HRQOL).

1.2 Long-term sequelae of SARS-CoV-2 infection in children and adolescents

The long-term impact of SARS-CoV-2 infection extends beyond symptoms described during the acute phase, affecting physical, psychological, and social domains. As the pandemic progressed, studies demonstrated the multisystemic nature of the disease, often associated with fatigue, chronic inflammation, and reduced HRQOL (33,34).

Potential mechanisms contributing to the pathophysiology of post-acute COVID-19 include virus-specific pathophysiologic changes, immunologic aberrations, and inflammatory damage in response to the acute infection, and the expected sequelae of post-critical illness (35). These mechanisms may be particularly relevant in children and adolescents with preexisting conditions or those diagnosed with MIS-C, as they could experience more pronounced or prolonged long-term side effects. Additionally, children and adolescents from vulnerable socioeconomic contexts face compounded challenges, as psychosocial stressors such as prolonged quarantine measures, isolation, family health concerns, and hospitalizations contribute to heightened anxiety and diminished HRQOL in this population (36–38).

A multinational, multicenter cohort study conducted across Europe provided valuable insights into the clinical presentation and outcomes of pediatric COVID-19 cases. The study found that while COVID-19 is generally a mild disease in children, a notable proportion required hospitalization, and some developed severe complications necessitating intensive care (6). Another cohort study found that 43% of pediatric patients with preexisting chronic conditions reported at least one persistent symptom between three to six months post-infection, and 25% experienced symptoms lasting beyond nine months. Additionally, these patients demonstrated impairments in physical and school domains of HRQOL, as assessed by the Pediatric Quality of Life Inventory (PedsQL™ 4.0) (39).

The long-term impact of MIS-C is also an area of concern. Penner et al. (2021) evaluated 46 patients diagnosed with MIS-C six months after hospital discharge and identified persistent sequelae, including muscle fatigue, proximal myopathy, anxiety, and emotional lability (40). Similarly, D'Auria et al. (2024) found that MIS-C patients experienced significant reductions in physical and psychological well-being, showing signs of persistent fatigue, emotional challenges, and functional impairments, even after one year of follow-up (41). Altogether, these findings underscore the critical need for a multidisciplinary follow-up approach to address physical, psychological, and functional aspects of recovery, ultimately aiming to mitigate long-term consequences and improve HRQOL in COVID-19 and MIS-C patients.

Another factor that may have amplified the side effects of the infection and the pandemic was the increase in physical inactivity caused by lockdowns and quarantine measures implemented to prevent the spread of the virus. It is well known that sedentary behavior (excessive sitting) and physical inactivity (low levels of moderate to vigorous physical activity) are associated with the incidence of various chronic diseases, such as coronary artery disease, acute myocardial infarction, hypertension, type II diabetes, and osteoporosis. These factors are also independently linked to increased mortality from all causes and can contribute to childhood obesity (42,43). In general, the maintenance of elevated levels of inactivity exacerbates the overall health status in youth with preexisting chronic conditions, potentially leading to the development of other chronic diseases and further deterioration of their clinical condition. This link between an inactive and sedentary behavior and the presence of preexisting disease creates a vicious cycle, that ultimately worsens the individual’s general health (44).

As a result, the need to adapt conventional exercise training methods/protocols to online platforms emerged, ensuring that children and adolescents, in particular, could access specialized programs even during periods of isolation. It is also important note that children who have had COVID-19 or have immunosuppressive conditions may remain isolated for longer periods, and those who develop complications related to the infection may take even more time to return to their daily activities (45).

The chronic course of both conditions resulting from SARS-CoV-2 infection can negatively impact HRQOL of children and adolescents, potentially leading them to a state of hypoactivity, that can be motivated by various factors (e.g., fear of future complications or reinfection) (36,39,46,47). This scenario further exacerbates physical inactivity and sedentary behavior among children and adolescents, who, according to major health organizations (48,49) already exhibits high levels of physical inactivity.

Thus, the use of digital tools and remote exercise programs can mitigate the negative effects of both pandemic and infection, by promoting health and well-being during periods of social distancing. In this context, a home-based exercise program emerges as an accessible therapeutic approach to counteract the impacts of sedentary behavior and physical inactivity associated with the COVID-19 pandemic.

1.3 Potential effects of exercise interventions after SARS-CoV-2 infection

A mentioned, restrictive measures, such as quarantine and social distancing, implemented to combat the COVID-19 pandemic significantly contributed to the escalation of physical inactivity, drastically altering the structure and nature of exercise routines. As a result, it became necessary to adapt conventional exercise training methods to online platforms to ensure that people, especially children and adolescents, could access exercise programs or activities even under lockdown restrictions.

It is well-established in the literature that digital tools and remote exercise programs have the potential to mitigate the negative effects of sedentary behavior, promoting health and well-being in the context of social distancing (50). Several studies suggested that physical activity interventions following COVID-19 infection can aid in recovery, improving both physical function

and mental well-being (51–53). Recent observational studies have highlighted the crucial role of physical activity in mitigating the effects of COVID-19 (54,55). Adults who engage in regular physical activity show an 11% lower risk of SARS-CoV-2 infection, a 36% reduction in the risk of hospitalization, a 34% decrease in disease severity, and a 43% reduction in COVID-19-related mortality compared to physically inactive individuals (56). Additionally, a dose-response relationship has been observed, with the greatest benefits seen in those engaging in at least 150 minutes of moderate-intensity exercise or 75 minutes of vigorous exercise per week (equivalent to 500 MET-minutes per week) (56,57). These findings emphasize the importance of physical activity as a public health strategy, not only for preventing severe COVID-19 complications but also for aiding recovery in affected patients.

In general, exercise is widely recognized as a powerful non-pharmacological strategy in preventing and managing various non-communicable diseases, as well as improving physical, psychological, and social well-being. Among its numerous advantages, exercise plays a crucial role in modulating the immune system, reducing inflammation across a range of chronic conditions (58–60) and lowering the incidence of viral infections (61). Furthermore, exercise directly contributes to an enhanced quality of life by improving overall well-being and promoting better mental health outcomes (62,63), including in children and adolescents (64,65).

2. OBJECTIVES

2.1 Main objective

The main goal of this thesis was to advance the understanding of the short- and long-term impacts of SARS-CoV-2 infection—including MIS-C—on children and adolescents, and to evaluate the effects of a 12-week home-based exercise training program on HRQOL.

2.2 Specific objectives

The specific objectives of this thesis were designed to investigate the effects of SARS-CoV-2 infection and the proposed intervention on a broad range of secondary health outcomes in pediatric patients, including cardiovascular, pulmonary, metabolic, and inflammatory parameters, as the followings:

I. To evaluate the impacts of COVID-19 and MIS-C on cardiorespiratory fitness, endothelial function, and cardiac function of children and adolescents with and without preexisting chronic conditions.
II. To assess the effects of 12-wk, home-based exercise program on cardiorespiratory capacity, endothelial and cardiac function and metabolic and inflammatory markers in children and adolescents following COVID-19 and MIS-C.

2.3 Hypothesis

Previous studies have demonstrated that HBET programs can improve physiological outcomes, including cardiorespiratory fitness, endothelial function, and overall HRQOL in children and adolescents. We then hypothesized that similar benefits can be achieved in our cohort of pediatric patients recovering from COVID-19 and MIS-C. Specifically, HBET is expected to mitigate the negative effects of sedentary behavior, quarantine measures and enhance physical and mental health outcomes in this population. This is particularly important for those with preexisting or immunocompromised conditions.

Moreover, assessing the health status of immunocompromised patients, including those recovering from the infection is essential for understanding the broader implications of the disease on this vulnerable population. This approach will help identify persistent physiological impairments and disparities in their recovery, providing evidence to support targeted rehabilitation strategies and preventive interventions.

3 METHODOLOGY

The results of this thesis have already been published and divided into four different articles, all accepted in international journals. Our findings are part of a broader institutional project titled “Prospective studies in schoolchildren and adolescents with COVID-19 treated at HCFMUSP” (see in Annex 1).

Therefore, this document is presented as a compilation of articles, structured according to the international academic standards required by FMUSP (66). Each study was conducted with methodological rigor and ethical compliance, adhering to established guidelines for research in pediatric populations. We included two cross-sectional analyses that investigated the impacts of COVID-19 (Article 1) and MIS-C (Article 2) on cardiovascular and pulmonary health, as well as the main clinical manifestations resulting from the infection in patients treated at the Hospital das Clínicas da Universidade de São Paulo. Subsequently, a case series (Article 3) was conducted to evaluate the effects of HBET program after MIS-C, exploring its applicability and the potential benefits for the overall health of these individuals. Finally, a randomized controlled trial (Article 4) was carried out to assess the effects of the same intervention on HRQOL and various physiological parameters in pediatric COVID-19.

Additionally, a year research internship was included as a part of the PhD project development, financed by the São Paulo Research Foundation. From July 2023 to August 2024, I contributed to significant research projects at the University of Washington (UW), Seattle Children's Hospital (SCH), and Boston Children’s Hospital (BCH). At UW, under the supervision of Prof. Jonathan A. Drezner, I contributed to epidemiological studies on cardiovascular health in athletes, leading a comprehensive analysis on sudden cardiac arrest among young competitive athletes in the United States (Astley, C., Petek, B. J., Delong, R. N., *et al.* "COVID-19 Did Not Increase the Risk of Sudden Cardiac Arrest Among Young Competitive Athletes in the United States", accepted for publication at *JAMA Network*). Additionally, we performed all the echocardiogram analysis related to the randomized controlled trial and prepared the final manuscript (Article 4).

At SCH, under the supervision of Prof. Michael Portman, my activities included managing research protocols, maintaining detailed records in the REDCap system, and supporting the conduct of national studies, such as the investigation of myocarditis and pericarditis associated with the COMIRNATY vaccine in individuals under 21 years of age. I also participated in a

protocol focused on DNA collection to investigate the genetic basis of Kawasaki disease, where I was responsible for obtaining participant consent, collecting biological samples, organizing reports, and managing clinical data. This work, sponsored by the National Institute of Health (NIH), is led by Dr. Portman.

My time at BCH was particularly enriching. Under the supervision of Dr. Naomi Gauthier, I had the opportunity to learn from cardiologists and exercise physiologists actively engaged in the prestigious Cardiac Fitness Program. This program is internationally recognized for its excellence and pioneering approach to developing personalized strategies for cardiac rehabilitation in children and adolescents with congenital heart disease and other rare cardiac conditions. I gained knowledge on cardiac rehabilitation protocols tailored to each patient's clinical condition, combining detailed assessments of cardiorespiratory fitness and electrophysiological aspects, leading to personalized interventions aimed at improving quality of life and diverse clinical outcomes.

Furthermore, I contributed to the analysis of a project evaluating the impact of mindset (fixed vs. growth mindset theory) on the physical performance of children with congenital heart disease. I also performed retrospective analyses of quantitative data from the program, enhancing my skills in interpreting cardiopulmonary exercise tests. In addition to academic activities, I attended seminars, workshops, and international conferences, including the oral presentation related to article 4 at the American College of Sports Medicine (ACSM) in Boston, MA. The work was submitted and accepted for publication in *Medicine & Science in Sports & Exercise*. My international experience provided comprehensive clinical research training, with a focus on pediatric cardiology and exercise physiology, strengthening my technical skills and consolidating my ability to collaborate within multidisciplinary teams.

4. PUBLISHED ARTICLES

The following sections correspond to each published article. Articles 1, 2, and 3 are open access and are fully detailed below. As Article 4 was published in a non-open-access journal, its results are summarized in the current document.

4.1 Article 1: Impaired cardiorespiratory fitness and endothelial function after SARS-CoV-2 infection in a sample of mainly immunocompromised youth

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RESEARCH ARTICLE

Long-Term Recovery from SARS-CoV-2 (COVID-19)

Impaired cardiorespiratory fitness and endothelial function after SARS-CoV-2 infection in a sample of mainly immunocompromised youth

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Abstract

This study aimed to compare cardiopulmonary fitness and endothelial function 6 months after hospital diagnosis in a sample mainly comprising immunocompromised patients with confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection versus noninfected controls. Youth (n = 30; age: 14 yr; 60% females) with confirmed SARS-CoV-2 seen in a tertiary hospital of Sao Paulo, Brazil, were matched by propensity score based on BMI, age, sex, and pre-existing diseases with a control group who had not been tested positive for SARS-CoV-2 infection (n = 30; age: 15 yr; 50% females). Cardiopulmonary fitness (by means of a cardiopulmonary exercise test: CPET) and brachial flow-mediated dilation (%b-FMD) were assessed 3–6 mo after diagnosis. Patients were matched by propensity score based on BMI, age, sex and pre-existing diseases, if any, with a control group who had not been tested positive for SARS-CoV-2. Compared with controls, patients with COVID-19 showed reduced ventilatory anaerobic threshold (VAT) and peak exercise time and minute ventilation/maximum voluntary ventilation ($\dot{V}_E/MVV$) (all P < 0.01). Brachial endothelial function variables were all adjusted for body surface area (BSA). Patients with COVID-19 had decreased %b-FMD (3.6 vs. 5.4; P = 0.03) mean and positive flow (P = 0.02 and P = 0.03, respectively) versus controls. Adjusted linear regression models exploring associations between CPET variables, %b-FMD and the potential predictors post-COVID-19 syndrome, number of symptoms, hospitalization, and COVID severity did not detect significant associations, except for total shear rate in hospitalization (coefficient: —65.07 [95%CI —119.5;—10.5], P = 0.02). Immunocompromised and previously healthy children and adolescents with COVID-19 presented with impaired exercise capacity and endothelial dysfunction when compared with their noninfected counterparts, but the mechanisms remain unknown.

NEW & NOTEWORTHY COVID-19 appeared to impair recovery of exercise capacity and endothelial function in a sample mainly comprising immunocompromised patients, but the mechanisms are unknown. These findings support the need for preventive measures against COVID-19 in this vulnerable population and suggest the necessity of proper monitoring and treatment for these patients.

COVID-19; cardiovascular health; exercise testing; pediatrics

INTRODUCTION

COVID-19 is a respiratory infectious disease with multisystemic involvement. In adults, it can negatively impact cardiorespiratory fitness, endothelial function, and overall quality of life (1–5). Despite children and adolescents being less likely to have severe COVID-19, pre-existing diseases are associated with worse prognosis, including those leading to immune impairment such as obesity, diabetes, hypertension, chronic kidney, lung, and heart diseases (6). Previously, we reported persistent symptoms and quality of life in a cohort of pediatric, immunocompromised patients following COVID-19 diagnosis. We observed that 43% of these patients reported at least one persistent symptom 3 to 6 months after infection, and 25% had persistent symptoms for at least 9 months. As compared with controls, patients with COVID-19 had often presented with hypoactivity and worse health-related quality of life (7).

COVID-19 has been associated with physical inactivity and deconditioning in different populations, which could be

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particularly problematic for diseased individuals (8, 9). In fact, children and adolescents with chronic conditions often present with hypoactivity, which can lead to a reduction in functional ability, poor cardiorespiratory capacity, and increased cardiovascular risk factors (9, 10). Cardiopulmonary (or aerobic) fitness (assessed by an incremental exercise test) is generally impaired in several pediatric chronic conditions (11). For example, in children and young adults with congenital heart or lung disease, a low aerobic fitness predicts morbidity and mortality in later years (12–14). One could hypothesize that COVID-19 could further deteriorate aerobic fitness in immunocompromised youth.

Endothelial function is another important marker of cardiovascular health that may be affected by COVID-19. The impairment of endothelial cells lining blood vessels can be related to mechanical damage, bacterial infection, hyperinflammation, and autoantibodies (15, 16). Endothelial function by flow-mediated dilatation can be informative of early-stage atherosclerosis (17). In fact, some studies have shown reduced endothelial function in children and adolescents at risk of atherosclerotic cardiovascular disease (17, 18). Importantly, time spent in sedentary behavior is considered the main predictor for lower arterial compliance in children (19). However, whether COVID-19 is detrimental to endothelial function in immunocompromised youth remains unknown.

In this exploratory study, we hypothesized that COVID-19 infection could impair the recovery of exercise capacity and the endothelial function. To test this hypothesis, we compared cardiopulmonary fitness and brachial flow-mediated dilation (%b-FMD) by means of a cardiopulmonary exercise test (CPET) in a sample mainly comprising immunocompromised patients with confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection versus noninfected controls. We also evaluated the association between the post-COVID-19 syndrome, number of symptoms, hospitalization, and COVID severity with cardiopulmonary fitness and endothelial function.

MATERIALS AND METHODS

This is a part of a longitudinal prospective study from our tertiary Hospital (Hospital das Clínicas da Universidade de São Paulo, HCFMUSP), aimed to explore the long-term effects of SARS-CoV-2 infection in surviving pediatric COVID-19 patients from a tertiary Latin America Hospital. The study was approved by the National and Institutional Ethical Committee of Clinical Hospital, CAAE: 37460620.8.0000.0068. Patients and guardians signed an informed consent before participants' enrollment.

Patients with laboratory-confirmed SARS-CoV-2 were invited by phone calls ~4 months after diagnosis. The inclusion criteria were: *1*) patients with SARS-CoV-2 infection confirmed by analyzing nasopharyngeal or oropharyngeal swabs using a genomic RNA assay with qRT-PCR or by anti-SARS-CoV-2 IgG serology and/or rapid immunochromatographic assay for anti-SARS-CoV-2 IgM and IgG antibodies and 2) age at diagnosis between 7 to 18 years old. The exclusion criteria were: *1*) presence of any physical limitation or disability that prevents the execution of the tests; *2*) pregnancy; *3*) patients diagnosed with Multisystem Inflammatory Syndrome (MIS-C); and *4*) patients with myocardial dysfunction, refractory cardiac arrhythmias, coronary artery aneurysms with or without thrombi, electrocardiographic changes suggestive of myocardial infarction or ischemia, and clinical signs of heart failure at the start of the study. We invited patients from our hospital clinics for the control group. In addition, siblings and friends of our patients with COVID-19 with no pre-existing diseases were also invited to participate. The inclusion criteria included: negative RT-PCR for SAR-CoV-2 and nonreactive serology for the detection of antibodies against SARS-CoV-2 and age varying between 7 and 18 yr at the time of inclusion in the study. The exclusion criteria were the same as COVID-19 group. Patients were recruited from October 2020 to August 2021 and the time between diagnosis and study entry was: 4 months (range: 0.7–6.6 months).

Patients' data during the acute phase were retrospectively obtained through medical records. Post-infection and subsequent visits were collected prospectively in an outpatient clinic for COVID-19 at HCFMUSP. All participants underwent a thorough assessment including demographic and anthropometric data, clinical manifestations assessed by dichotomous questions (yes and no), namely fever, nasal discharge, sneezing, cough, sore throat, anosmia (loss of smell), dysgeusia (taste alterations), headache, myalgia (muscle pain), arthralgia (joint pain), conjunctivitis, dyspnea, hypoxemia (low oxygen in blood), nausea, vomiting, diarrhea, cutaneous rash, neurological abnormalities, and hospitalization. COVID-19 was classified according to clinical features and chest X-ray imaging, being defined as mild, moderate, severe, and critical cases, as follows: mild: symptoms of acute upper respiratory tract infection; Moderate: patients with pneumonia, without need of oxygen therapy; and Severe: patients with pneumonia, dyspnea, with cyanosis and oxygen saturation below 92%; critical, acute respiratory distress syndrome or respiratory failure, could also present with organ dysfunction. Post-COVID-19 syndrome was defined as ongoing symptoms 12 wk or more after acute COVID-19 (20).

Pre-existing pediatric chronic conditions in children and adolescents with COVID-19 and controls were defined according to the duration of signs and symptoms greater than three months, and the diagnosis was established for each disease based on the physician's scientific knowledge and diagnostic criteria (21, 22).

Cardiopulmonary Exercise Test

CPET was performed on a treadmill with an intensity-graded, maximal effort protocol and continuous gas exchange measurement (Metalyzer IIIb/breath-by-breath, Cortex Leipzig Germany). Test termination was determined by volitional exhaustion, and maximal effort was confirmed by a peak respiratory exchange ratio $\geq$1.0, maximal heart rate > 95% age/gender-predicted values, or maximum rating of perceived exertion (RPE) (23).

To assess ventilatory efficiency and capacity, we measured ventilation-to-maximum voluntary ventilation ratio ($\dot{V}_E$/MVV), the highest value for end-tidal carbon dioxide pressure ($P_{ET_{CO_2}}$ max mmHg), peak O_2 pulse (mL/beat), and heart rate (HR) recovery 1 min after exercise (beats/min).

Other CPET variables such as oxygen consumption at ventilatory anaerobic threshold ($\dot{V}_{O_2}$ VAT), peak oxygen consumption ($\dot{V}_{O_{2peak}}$), oxygen uptake efficiency slope (OUES)

(L/min), the lowest $\dot{V}_E/\dot{V}_{CO_2}$ ratio, $\dot{V}_E/\dot{V}_{CO_2}$ slope, and respiratory exchange ratio (RER) were analyzed as previously described (23). For all dependent variables, reference values (sorted by age and sex) from healthy children were used whenever available (24, 25).

Brachial Flow-Mediated Dilation

%b-FMD was evaluated according to current guidelines (26) using a high-resolution ultrasound machine (LOGIQ e PRO – GE Healthcare, Chicago, IL) equipped with a 4.0–12.0 MHz linear transducer. Initially, participants were positioned in the supine position with their right arm extended at an angle of ~80° from the torso. Longitudinal images of the brachial artery diameter were taken using the B-mode ultrasound, and simultaneous pulse-waved Doppler blood flow velocity was obtained using a 60° insonation angle with the sample volume placed in mid-artery and aligned with the blood flow. Initially, a 1-min baseline recording of the brachial artery diameter and blood flow velocity was performed. Then, the ischemic stimulus was performed by inflating a cuff placed in the forearm to 60 mmHg above the patient's resting systolic pressure for 5 min. Recordings were resumed 60 s before cuff deflation and continued for 3 min thereafter. Brachial artery diameter and shear rate (4 × mean blood velocity/internal diameter) were analyzed by a blinded evaluator using a semi-automatic edge-detection and wall-tracking software (Cardiovascular Suite, Quipu, Pisa, Italy). %b-FMD was calculated as the percentage change of the brachial artery diameter after cuff release in relation to baseline brachial artery diameter [b-FMD = (baseline diameter – peak diameter/baseline diameter) × 100]. Blood flow was calculated as the product of the average flow velocity and vessel radius (Flow = Vm × πr^2 × 60). Antegrade and retrograde components of blood flow were also computed, representing the area above and below the baseline of the horizontal Doppler axis, respectively. %b-FMD lower than the age- and sex-specific 25th percentile (27) was considered as suggestive of endothelial dysfunction. In addition, all ultrasound data were corrected by normalizing it to body surface area (BSA).

Statistical Analysis

Participants (n = 68) were matched for BMI, age, sex, and pre-existing diseases using propensity scores. Matching was achieved through the R package "MatchIt" (28), utilizing optimal pair matching and logistic regression to calculate propensity scores, resulting in 30 matched individuals per group. Then, descriptive statistics were used to summarize participants' characteristics. Continuous variables were described as means ± standard deviation (SD), and categorical variables were presented as absolute (n) and percentage values (%). Between-group variables were analyzed using either Student's t or Mann-Whitney test for continuous variables and Fisher's exact test for categorical variables. The association between the post-COVID-19 syndrome, number of symptoms, hospitalization, and COVID severity with cardiopulmonary fitness and endothelial function were tested using linear regression models. Post-COVID-19 syndrome (Yes or No), number of symptoms (0 to 13), hospitalization (Yes or No) and COVID severity (1 to 3) were the independent variables, and CPET variables (e.g., time at VAT and peak, $\dot{V}_{O_2}$ VAT and $\dot{V}_{O_2}$ peak, O_2 pulse peak, OUES·kg^{-1}, $\dot{V}_E$ at peak exercise, $\dot{V}_E$/MVV, and $\dot{V}_E/\dot{V}_{CO_2}$ slope) and %b-FMD variation were the dependent variables. An α of 0.05 was previously set for all analyses. Results are presented as the coefficient from the respective model (i.e., the average change in the dependent variable when the independent variable increases by 1 unit), alongside their respective P values and 95% confidence intervals. Statistical test, regression analysis, and visualizations were done in R (version 4.2.2, R Core Team, Vienna, Austria), using the "stats" and "ggplot2" packages (29).

RESULTS

Patients with COVID-19 with and without pre-existing conditions were included in this cohort. The noninfected control group was deemed similar regarding pre-existing diseases, sex, age, and body mass index (BMI) (all $P > 0.05$). The main characteristics, symptoms, and current therapy during acute phase are shown in Table 1. The most frequent symptoms at

Table 1. *Characteristics of participants*

Variables	COVID-19 (n = 30)	Control (n = 30)
Age at diagnosis, yr	14 (8–18)	15 (8–17)
Female sex, n (%)	18 (60)	15 (50)
Weight, kg	50 ± 19	51 ± 14
Height, m	1.5 ± 0.2	1.5 ± 0.13
Body mass index, kg·m^{-2}	21 ± 5	21 ± 4
Body surface area, m^2	1.5 ± 0.3	1.5 ± 0.2
Symptoms, n (%)		
Fever	16 (53)	
Nasal discharge, n (%)	14 (46)	
Sneezing, n (%)	4 (13)	
Cough, n (%)	9 (30)	
Sore throat, n (%)	8 (26)	
Anosmia, n (%)	7 (26)	
Dysgeusia, n (%)	5 (16)	
Headache, n (%)	12 (40)	
Myalgia, n (%)	7 (23)	
Arthralgia, n (%)	3 (10)	
Dyspnea, n (%)	8 (27)	
Hypoxemia, n (%)	1 (3)	
Nausea, n (%)	7 (23)	
Vomiting, n (%)	5 (17)	
Diarrhea, n (%)	5 (17)	
Abdominal pain, n (%)	3 (10)	
Neurological symptoms, n (%)	3 (10)	
Hospitalization	8 (27)	
Length of hospital stay, days	3.5 (1-11)	
Post-COVID symptoms, n (%)	10 (36)	
Pediatric pre-existing chronic diseases		
Autoimmune rheumatic and inflammatory diseases	16 (53)	13 (43)
Hepatic and kidney diseases	7 (23)	7 (23)
Oncological diseases	1 (3)	1 (3)
Pulmonary diseases	1 (3)	5 (16)
Previously health	5 (16)	4 (13)
Pharmacological therapy		
Prednisone	4 (13)	3 (10)
Biologic	7 (23)	3 (10)
Immune-system suppressant	3 (10)	6 (20)
Pediatric COVID-19 classification		
Mild	23 (77)	
Moderate	5 (17)	
Severe	2 (6)	

Results are presented in n (%), median (minimum-maximum values), or means ± SD.

the acute phase were fever (53%), followed by nasal discharge (46%), headache (40%), cough (30%), and myalgia (23%). Also, 32% of our patients were hospitalized and 36% showed post-COVID-19 symptoms 6 months after hospital discharge. Most of our patients (77%) had mild COVID-19, whereas 17% and 6% had moderate and severe disease classification. Fourteen patients (47%) in the COVID-19 group and twelve (40%) in the control group were undergoing current pharmacologic therapy, with no discernible difference between the two groups.

CPET and %b-FMD parameters are shown in Table 2. Compared with controls, patients with COVID-19 showed reduced VAT exercise time (3.6 vs. 5.4; $P < 0.01$), peak exercise time (9.8 vs. 11.8; $P < 0.01$) and $\dot{V}_E$/MVV (0.5 vs. 0.7; $P < 0.01$). Regarding endothelial function, patients with COVID-19 had decreased %b-FMD (3.6 vs. 5.4; $P = 0.03$) mean and positive flow ($P = 0.02$ and $P = 0.03$, respectively) compared with controls. Adjusted linear regression models exploring associations between CPET variables, %b-FMD and the potential predictors post-COVID-19 syndrome, number of symptoms, hospitalization, and COVID severity did not detect significant associations, except for total shear rate in hospitalization (coefficient: −65.07 [95%CI −119.5; −10.5], $P = 0.02$) (Fig. 1).

DISCUSSION

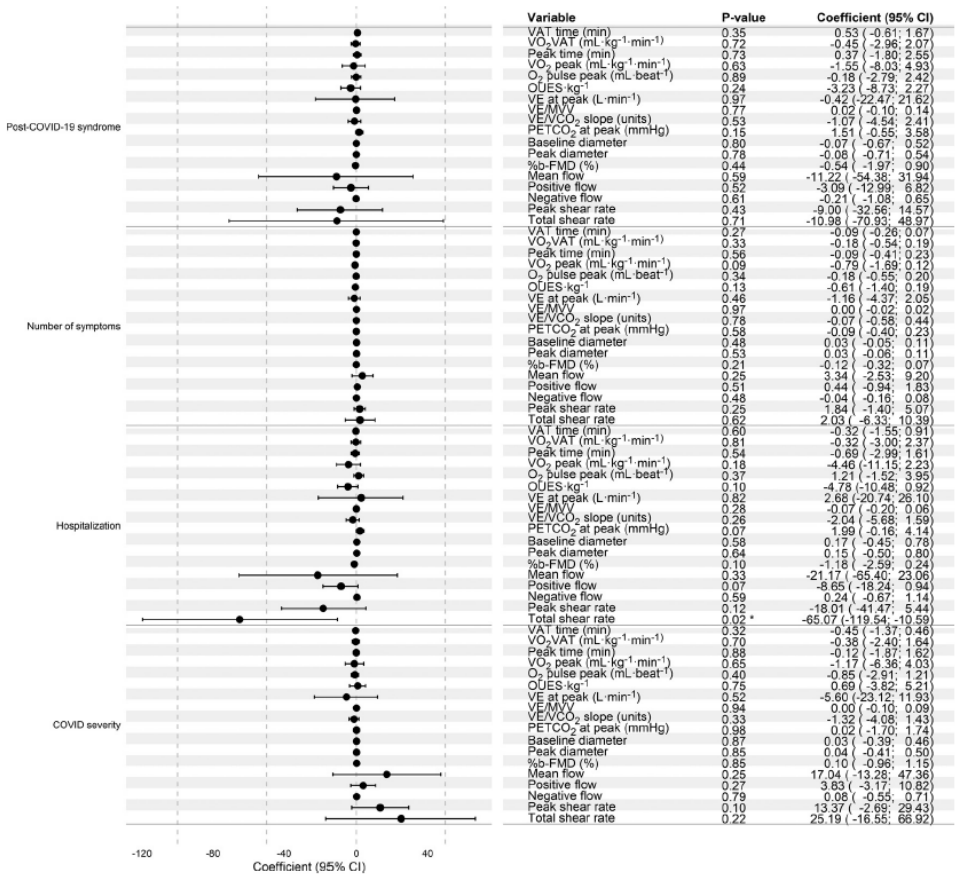
To the best of our knowledge, this is the first study to describe abnormalities in cardiorespiratory fitness and endothelial function in a sample of mainly immunocompromised youth after SARS-CoV-2 infection. Our findings were independent of hospitalization, number of symptoms, and disease severity during the acute phase.

We observed that, compared with controls, our patients with COVID-19 exhibited diminished VAT exercise time and time to exhaustion, as indicative of an exercise when compared with controls. $\dot{V}O_{2peak}$ is the most frequently CPET parameter used to assess aerobic fitness as it has a great predictable value for morbidity and mortality (30). Reduced $\dot{V}O_{2peak}$ has been reported in adults with COVID-19 (3, 5, 31). For instance, Longobardi et al. reported reduced $\dot{V}O_{2peak}$ in adults with severe COVID-19 (32). At least in adults, the mechanisms underlying the COVID-19-induced reduction in $\dot{V}O_{2peak}$ seem to be multifactorial, but predominately periphery (e.g., impaired oxygen extraction by the skeletal muscle disturbances, altered muscle metabolism, and compromised $\dot{V}O_2$ kinetics) (32, 33). In our sample, the difference in $\dot{V}O_2$ between groups was marginal (~6%) and did not reach statistical significance, which suggests that this parameter could be less affected in pediatric populations than in adults. The altered VAT time observed herein supports the notion that COVID-19 may disrupt aerobic metabolism, a finding already shown in adults with severe COVID-19 (32). Unraveling the factors are responsible for reduced aerobic fitness and exercise intolerance in immunocompromised children and adolescents with COVID-19 require further studies.

Table 2. *Cardiopulmonary exercise test and brachial flow-mediated dilation in patients with COVID-19 vs. control group*

Variables Circulation	Reference Values	COVID-19	Control	*P* Value
Resting heart rate, beats/min		100.1 ± 17.9	95.3 ± 13.2	0.608
Peak heart rate, beats/min		183.7 ± 16.1	184.7 ± 14.7	0.846
Heart rate 1 min recovery, beats/min		169.2 ± 22	165.9 ± 17.3	0.576
Performance				
Respiratory exchange ratio	1.1	1.1 ± 0.1	1.1 ± 0.1	0.451
VAT exercise time, min		3.6 ± 1.4	5.4 ± 1.4	<0.001*
$\dot{V}O_2$ VAT, mL·kg^{-1}·min^{-1}		14.9 ± 3.1	15.9 ± 3.9	0.508
Peak exercise time, min		9.8 ± 2.7	11.8 ± 2.7	0.004*
$\dot{V}O_{2peak}$, mL·kg^{-1}·min^{-1}		29.3 ± 8	31.6 ± 7.1	0.226
% Predicted $\dot{V}O_{2peak}$	<80%	59.5 ± 13.6	64.5 ± 13.4	0.318
Ventilatory and gas exchange response				
Peak O_2 pulse, mL/beat		8.6 ± 3.2	8.9 ± 1.6	1
OUES, units/kg	35	30.9 ± 6.9	32.5 ± 7.5	0.582
$\dot{V}_E$ at VAT, L/min		21.4 ± 6.2	23.9 ± 5.2	0.064
$\dot{V}_E$ at peak, L/min		63.1 ± 27	69.8 ± 13.9	0.129
$\dot{V}_E$/MVV, L/min		0.5 ± 0.2	0.7 ± 0.1	0.002*
$\dot{V}_E/\dot{V}CO_2$ slope, units	31	34.5 ± 4.3	37 ± 4.2	0.06
$P_{ET_{CO_2}}$ max, mmHg	35	35.3 ± 2.6	33.3 ± 3.4	0.071
Chronotropic reserve		95.1 ± 19.4	95.2 ± 15.7	0.807
Doppler ultrasound of the brachial artery				
Basal diameter, mm		2.3 ± 0.6	2.4 ± 0.7	0.710
Peak diameter, mm		2.5 ± 0.6	2.6 ± 0.6	0.405
%b-FMD, %		3.6 ± 1.5	5.4 ± 3.2	0.035*
Shear rate total, $_{AUC}$		98073.2 ± 60541.3	129452 ± 78684.6	0.112
Shear rate peak, $_{AUC}$		34240 ± 24100	35268.1 ± 15402.3	0.346
Mean flow, mL/min		94.7 ± 43.8	186.3 ± 212.4	0.020*
Positive flow, mL/min		20.5 ± 10.1	30.9 ± 23.1	0.039*
Negative flow, mL/min		−0.8 ± 0.9	−1.4 ± 1.5	0.089

Results are presented in means ± SD. VAT, Ventilatory anaerobic threshold; $\dot{V}O_2$, oxygen uptake; OUES, oxygen uptake efficiency slope; $\dot{V}_E$, minute ventilation; MVV, maximum voluntary ventilation; $\dot{V}_E/\dot{V}O_2$, ventilatory equivalent for carbon dioxide production; $\dot{V}_E/\dot{V}CO_2$, carbon dioxide production; $P_{ET_{CO_2}}$, end-tidal carbon dioxide tension; b-FMD, brachial flow-mediated dilation; AUC, area under curve. *$P < 0.05$ from Mann–Whitney test.



Variable	P-value	Coefficient (95% CI)
VAT time (min)	0.35	0.53 (-0.61; 1.67)
VO_2VAT (mL·kg^{-1}·min^{-1})	0.72	-0.45 (-2.96; 2.07)
Peak time (min)	0.73	0.37 (-1.80; 2.55)
VO_2 peak (mL·kg^{-1}·min^{-1})	0.63	-1.55 (-8.03; 4.93)
O_2 pulse peak (mL·beat^{-1})	0.89	-0.18 (-2.79; 2.42)
OUES·kg^{-1}	0.24	-3.23 (-8.73; 2.27)
VE at peak (L·min^{-1})	0.97	-0.42 (-22.47; 21.62)
VE/MVV	0.77	0.02 (-0.10; 0.14)
VE/VCO_2 slope (units)	0.53	-1.07 (-4.54; 2.41)
$PETCO_2$ at peak (mmHg)	0.15	1.51 (-0.55; 3.58)
Baseline diameter	0.80	-0.07 (-0.67; 0.52)
Peak diameter	0.78	-0.08 (-0.71; 0.54)
%b-FMD (%)	0.44	-0.54 (-1.97; 0.90)
Mean flow	0.59	-11.22 (-54.38; 31.94)
Positive flow	0.52	-3.09 (-12.99; 6.82)
Negative flow	0.61	-0.21 (-1.08; 0.65)
Peak shear rate	0.43	-9.00 (-32.56; 14.57)
Total shear rate	0.71	-10.98 (-70.93; 48.97)
VAT time (min)	0.27	-0.09 (-0.26; 0.07)
VO_2VAT (mL·kg^{-1}·min^{-1})	0.33	-0.18 (-0.54; 0.19)
Peak time (min)	0.56	-0.09 (-0.41; 0.23)
VO_2 peak (mL·kg^{-1}·min^{-1})	0.09	-0.79 (-1.69; 0.12)
O_2 pulse peak (mL·beat^{-1})	0.34	-0.18 (-0.55; 0.20)
OUES·kg^{-1}	0.13	-0.61 (-1.40; 0.19)
VE at peak (L·min^{-1})	0.46	-1.16 (-4.37; 2.05)
VE/MVV	0.97	0.00 (-0.02; 0.02)
VE/VCO_2 slope (units)	0.78	-0.07 (-0.58; 0.44)
$PETCO_2$ at peak (mmHg)	0.58	-0.09 (-0.40; 0.23)
Baseline diameter	0.48	0.03 (-0.05; 0.11)
Peak diameter	0.53	0.03 (-0.06; 0.11)
%b-FMD (%)	0.21	-0.12 (-0.32; 0.07)
Mean flow	0.25	3.34 (-2.53; 9.20)
Positive flow	0.51	0.44 (-0.94; 1.83)
Negative flow	0.48	-0.04 (-0.16; 0.08)
Peak shear rate	0.25	1.84 (-1.40; 5.07)
Total shear rate	0.62	2.03 (-6.33; 10.39)
VAT time (min)	0.60	-0.32 (-1.55; 0.91)
VO_2VAT (mL·kg^{-1}·min^{-1})	0.81	-0.32 (-3.00; 2.37)
Peak time (min)	0.54	-0.69 (-2.99; 1.61)
VO_2 peak (mL·kg^{-1}·min^{-1})	0.18	-4.46 (-11.15; 2.23)
O_2 pulse peak (mL·beat^{-1})	0.37	1.21 (-1.52; 3.95)
OUES·kg^{-1}	0.10	-4.78 (-10.48; 0.92)
VE at peak (L·min^{-1})	0.82	2.68 (-20.74; 26.10)
VE/MVV	0.28	-0.07 (-0.20; 0.06)
VE/VCO_2 slope (units)	0.26	-2.04 (-5.68; 1.59)
$PETCO_2$ at peak (mmHg)	0.07	1.99 (-0.16; 4.14)
Baseline diameter	0.58	0.17 (-0.45; 0.78)
Peak diameter	0.64	0.15 (-0.50; 0.80)
%b-FMD (%)	0.10	-1.18 (-2.59; 0.24)
Mean flow	0.33	-21.17 (-65.40; 23.06)
Positive flow	0.07	-8.65 (-18.24; 0.94)
Negative flow	0.59	0.24 (-0.67; 1.14)
Peak shear rate	0.12	-18.01 (-41.47; 5.44)
Total shear rate	0.02 *	-65.07 (-119.54; -10.59)
VAT time (min)	0.32	-0.45 (-1.37; 0.46)
VO_2VAT (mL·kg^{-1}·min^{-1})	0.70	-0.38 (-2.40; 1.64)
Peak time (min)	0.88	-0.12 (-1.87; 1.62)
VO_2 peak (mL·kg^{-1}·min^{-1})	0.65	-1.17 (-6.38; 4.03)
O_2 pulse peak (mL·beat^{-1})	0.40	-0.85 (-2.91; 1.21)
OUES·kg^{-1}	0.75	0.69 (-3.82; 5.21)
VE at peak (L·min^{-1})	0.52	-5.60 (-23.12; 11.93)
VE/MVV	0.94	0.00 (-0.10; 0.09)
VE/VCO_2 slope (units)	0.33	-1.32 (-4.08; 1.43)
$PETCO_2$ at peak (mmHg)	0.98	0.02 (-1.70; 1.74)
Baseline diameter	0.87	0.03 (-0.39; 0.46)
Peak diameter	0.85	0.04 (-0.41; 0.50)
%b-FMD (%)	0.85	0.10 (-0.96; 1.15)
Mean flow	0.25	17.04 (-13.28; 47.36)
Positive flow	0.27	3.63 (-3.17; 10.82)
Negative flow	0.79	0.08 (-0.55; 0.71)
Peak shear rate	0.10	13.37 (-2.69; 29.43)
Total shear rate	0.22	25.19 (-16.55; 66.92)

Figure 1. Association between post-COVID-19 syndrome, number of symptoms, hospitalization, and COVID severity with cardiorespiratory fitness and endothelial function.

Adults infected with severe COVID-19 and children with COVID-19-related multisystemic inflammatory syndrome (MIS-C) may be at higher risk for endothelial dysfunction, an important predictor of atherosclerosis (34). To our knowledge, this is the first study to assess endothelial function in immunocompromised youth following COVID-19. Our findings seem to suggest that this population may have impaired %b-FMD versus controls. It has been suggested that a residual activation of the immune system after the acute phase of COVID-19 may be involved in the endothelial dysfunction (1, 34). Furthermore, two recent studies pointed out that the alteration of endothelial barrier properties in systemic and pulmonary circulation may represent a key pathogenic mechanism of the reduced CPET performance in COVID-19 survivors (35, 36). However, it's worth noting that recent data also suggest that FMD, flow, and shear rate are not related to the severity of COVID-19 disease (37). Establishing the implications of this discovery is challenging, given that the natural progression of this subclinical condition remains uncertain. The repercussion of this finding, however, is difficult to establish as the natural course of this subclinical condition is still unknown. As such, monitoring patients with impaired endothelial function seems a reasonable approach to detect/prevent long-term cardiovascular complications appears to be reasonable.

The limitations of this study involve its cross-sectional design, which precludes causation; the relatively low sample investigated, which may have partially compromised the power of our regression models; the inability to distinguish the separate effects of COVID-19 and pre-existing conditions on the abnormalities observed herein; the lack of assessments to determine the mechanistic pathway of these alterations; the inability of extrapolating these data to other youth populations with a higher proportion of healthy individuals who are infrequently affected by persistent symptoms; the lack of assessment or control for the phase of the menstrual cycle for the female participants during data collection, which may have affected some of our findings; the lack of lifestyle assessments which may have impacted differently those who were versus those who were not infected (e.g., physical inactivity imposed by social isolation in the infected ones), and the inability to distinguish the effects of vaccines or different SARS-CoV-2 variants (which were not assessed) on the outcomes. In addition, the regression analyses were largely

exploratory, and due to the high number of statistical tests performed, type 1 error rates may have been inflated. As such, careful interpretation and further confirmation of these exploratory findings are necessary.

In conclusion, this study demonstrates that previously healthy and immunocompromised patients have a higher risk of impaired cardiorespiratory fitness and endothelial function 6 mo after SARS-CoV-2 infection as compared with noninfected controls, but the underlying mechanisms remain unknown.

DATA AVAILABILITY

Access to de-identified data or related documents can be requested through submission of a proposal with a valuable research question, necessary data protection plan, and ethical approvals. Data requests should be addressed to the corresponding author.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.A., M.F.B., H.H.M., A.M., C.A.S., and B.G. conceived and designed research; C.A., D.M.L.d.P., S.M.S., P.S., T.F., L.L., O.M., F.M., V.B., and G.N.L. performed experiments; C.A., D.M.L.d.P., S.M.S., G.P.E., L.L., O.M., F.M., V.B., and G.N.L. analyzed data; C.A., D.M.L.d.P., S.M.S., G.P.E., T.F., and C.A.S. interpreted results of experiments; C.A., G.P.E., and H.H.M. prepared figures; C.A., H.H.M., H.R., and B.G. drafted manuscript; C.A., P.S., T.F., L.L., O.M., F.M., V.B., M.F.B., H.H.M., A.M., C.A.S., H.R., and B.G. edited and revised manuscript; C.A., D.M.L.d.P., S.M.S., G.P.E., P.S., T.F., L.L., O.M., F.M., V.B., M.F.B., H.H.M., A.M., G.N.L., C.A.S., H.R., and B.G. approved final version of manuscript.

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4.2 Article 2: In-depth cardiovascular and pulmonary assessments in children with multisystem inflammatory syndrome after SARS-CoV-2 infection: A case series study

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ORIGINAL ARTICLE

Physiological Reports

In-depth cardiovascular and pulmonary assessments in children with multisystem inflammatory syndrome after SARS-CoV-2 infection: A case series study

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Abstract

We assessed PET-CT myocardial blood flow (MBF) using N-13 ammonia, brachial flow-mediated dilation, and cardiopulmonary exercise test in five post-discarged MIS-C survivors. None of the patients (median age: 9, range: 7-18 years; 3 females; 2 males) had preexisting pediatric chronic conditions. At the follow-up visit, two patients exhibited severe perfusion defect developed in the left ventricular cavity, suggesting extensive myocardial ischemia (MBF <2.0) and one patient showed persistent mild pericardial effusion. Others two patients demonstrated endothelial dysfunction. Nevertheless, all patients had lower predicted values in the VO_{2peak}, VO_{2VAT}, OUES, and O_2 Pulse (range: 35.2%–64.5%; 15.6%–38.2%; 1.0–1.3 L/min; 4–7 ml/beat), respectively. Our d suggested that previously health MIS-C patients had impaired MBF, endothelial dysfunction and lower cardiopulmonary capacity at follow-up analysis. Multidisciplinary further investigations should be conducted to reinforce these findings.

KEYWORDS

cardiovascular imaging, COVID-19, inflammation, MIS-C, children

1 | INTRODUCTION

Multisystem inflammatory syndrome in children (MIS-C) is a hyperinflammatory response that commonly develops within 2–6 weeks following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, requiring hospitalization in the acute phase (Ballin & Nordström, 2021). While multiple cohorts have shown that MIS-C patients may present with heterogeneous signs that include hemodynamic instability, tachycardia, left ventricular dysfunction, and respiratory distress, possibly primary or caused by cardiac dysfunction (Bateman et al., 2021; Bongers et al., 2011) the pathophysiology of MIS-C and its natural course are yet to be fully elucidated.

Herein we report on a broad, in-depth assessment of cardiovascular and pulmonary outcomes in a series of post discharged MIS-C survivors, which unravels novel pathological features associated with this syndrome.

2 | METHODS

2.1 | Study design and patients

This case series is part of a prospective cohort study aimed at exploring the spectrum of the long-term effects of COVID-19 and MIS-C in the pediatric population (clinicaltrials.gov NCT04659486). Data of the patients' acute phase were retrospectively assessed through medical records. The post-discharge data were collected prospectively in a dedicated, multidisciplinary, outpatient clinic for COVID-19 (n = 130) and MIS-C (n = 16) at the Children' and Adolescents' Institute of the Clinical Hospital of the University of Sao Paulo, between October 2020 and July 2021. Out of the 16 MIS-C patients followed at our clinic, 4 died, 4 did not meet inclusion criteria (3 were younger than 7 years and 1 had type I diabetes mellitus), and 3 did not accept to participate. Therefore, five patients were included. All patients (median age: 9, range: 7–18 years; 3 females) fulfilled the MIS-C diagnosis according to the Center for Disease Control (CDC) criteria (CDC, 2020). Four patients had positive serologic tests (ELISA assay to detect IgG for SARS-CoV-2), and one had a negative serologic test but was exposed to a confirmed COVID-19 case within 4 weeks prior to the onset of symptoms. None of the patients had any preexisting pediatric chronic conditions. Patients' main characteristics at hospital admission (during the acute phase) are shown in Table 1. Four out of five patients were admitted to the pediatric intensive care unit. Two patients required respiratory support and oxygen therapy, and three had vasodilatory shock. The median length stay was 12 (range: 3–18) days. The median time elapsed from discharge to the follow-up visit was 1.9 (range: 1.3–6.2) months. At the follow-up visit, we conducted a battery of assessments as follows: ^{13}N-ammonia PET-CT imaging, standard echocardiography, brachial flow-mediated dilation (FMD) using a Doppler ultrasound, maximal cardiopulmonary exercise test, and blood markers (C-reactive protein, D-dimer, fibrinogen, and troponin T). This study was approved by the local ethics committee (protocol #37460620.8.0000.0068) and registered at ClinicalTrials.gov (NCT04659486). Patients and guardians signed informed consent to participate in the study.

2.2 | ^{13}N-ammonia PET-CT imaging protocol

^{13}N-ammonia was produced by means of an on-site cyclotron installed at our institution (PETtrace™ 880; GE Healthcare), by ^{16}O(p,α) 13N. In this procedure, ^{13}N-ammonia is synthesized directly in the target water (in-target production) by adding ethanol 5 mmol as a free radical scavenger to prevent the formation of the oxo anions. The radiochemical purity was >99.9% within 60 min from the end of the bombardment. Subjects were maintained in a fasting state for at least 6 h before the study and were told not to consume methylxanthine-containing foods or beverages (coffee, chocolates, soft drinks and tea) for at least 24 h before the PET scan.

For measurement of myocardial blood flow (MBF) at rest and at pharmacological stress (adenosine-induced hyperemia), ^{13}N-ammonia was administered intravenously (0.286 mCi/kg) over a 10-s period, the intravenous line was flushed with additional saline over a 10-s interval and standardized imaging protocols were performed according to the American Society of Nuclear Cardiology guidelines (Vries et al., 2006). Stress imaging was performed identically, after adenosine infusion over 6 min (0.142 mg/min/kg). The myocardial perfusion radiopharmaceutical was injected about halfway into the adenosine infusion (at 3 min), when maximal vasodilatation and myocardial hyperemia were assumed to occur. MBF was expressed as ml/g/min.

The quantitative PET datasets were fused with CT using commercially available software (CardIQ Fusion, GE Healthcare). Quantitative MBF and myocardial flow reserve (MFR) was determined using the PMOD™ software package, version 3.4002 (PMOD Technologies LLC). Myocardial and blood-pool time-activity curves (TAC) were obtained from dynamic frames corrected for radioisotope decay. Segmental MBF was measured in each phase (rest and stress adenosine) by the model fitting of

TABLE 1 Clinical features among MIS-C patients

Patient's characteristics	Mean (SD), median (range) or *N* (%)	P1	P2	P3	P4	P5
Sex (female)	3 (60)	Female	Male	Female	Female	Male
Age (years)	10.2 (3.56)	16	7	9	8	11
Previously medical history	0 (0)	None	None	None	None	None
BMI (kg/m^2)	20.1 (3.51)	24.7	21.2	18.3	21.1	15.3
Height (cm)	140 (0.11)	156	126	145	135	138
Weight (kg)	40.0 (11.9)	60.3	33.7	38.6	38.4	29.3
Signs and symptons at admission						
Fever (days)	7.60 (4.72)	Yes (12)	Yes (8)	Yes (12)	Yes (1)	Yes (5)
Conjutivitis	3 (60)	Yes	Yes	No	No	Yes
Hypotension	4 (80)	Yes	Yes	Yes	No	Yes
Shock	3 (60)	No	Yes	Yes	No	Yes
Abdominal pain	5 (100)	Yes	Yes	Yes	Yes	Yes
Diarrhea	3 (60)	Yes	No	No	Yes	Yes
Treatment						
ICU admission	4 (80)	No	Yes	Yes	Yes	Yes
Length of stay at hospital (days)	10.4 (6.26)	3	14	18	5	12
Respiratory support/oxygen therapy	2 (40)	No/No	Yes/Yes	No/No	No/No	Yes/Yes
Anti-inflammatory treatment	2 (40)	No	Yes (mPRED)	Yes (mPRED)	No	No
Immunoglobulin treatment	5 (100)	First dose 2 g/kg	First dose and second dose 2 g/kg	First dose 2 g/kg	First dose 2 g/kg	First dose 2 g/kg
^{13}N-ammonia PET-CT						
Global MFR (abnormal when < <2), gray zone 2–2.5 and normal >2.5	2 (40)	1.6	3.7	3.2	1.8	2.5
Echo parameters						
Normal echocardiogram at follow-up	4 (80)	Normal	Normal	Normal	Abnormal[a]	Normal
LVDD *z*-score	−0.13 (1.01)	0.81	−0.74	0.1	−1.57	0.74
LVSD *z*-score	−0.66 (0.32)	−0.52	−0.83	−0.39	−0.43	−1.17
Septum *z*-score	0.91 (0.38)	0.55	1.3	0.56	0.84	1.33
LVPW *z*-score	0.62 (0.37)	0.21	1.00	0.31	0.58	1.00
LA *z*-score	−0.72 (1.07)	0.68	0	−0.78	−1.82	−1.7
LVEF (%) (abnormal ≤55)	1 (20)	75	70	70	54	79
Doppler ultrasound of the brachial artery						
FMD (%)	6.38 (3.41)	9.36	–	3.92	10.07	2.14
FMD reference value (25th percentile [Hadi et al., 2005])	6.05 (0.40)	5.92	–	6.19	6.23	5.36

(Continues)

TABLE 1 (Continued)

Patient's characteristics	Mean (SD), median (range) or *N* (%)	P1	P2	P3	P4	P5
Endothelial dysfunction (i.e., below the FMD 25th percentile [Hadi et al., 2005])	2 (50)	No	–	Yes	No	Yes
Cardiopulmonary exercise test						
VO_{2peak} (ml/g/min)	26.3 (8.35)	22.5	17.4	28.6	–	36.8
Predicted VO_{2peak} (%) (<80% abnormal)	50.2 (12.8)	48.7	35.2	64.5	–	52.7
VO_{2VAT} (ml/g/min)	13.7 (4.64)	7.2	12.4	17.0	–	16.9
% from expected value	−54.0 (14.5)	−70.4	−60.8	−37.3		−47.6
VO_{2VAT} (%)/predicted VO_2peak (<40 abnormal)	27.8 (9.73)	15.6	25.1	38.2	–	32.3
OUES (L/min)	1.20 (0.14)	1.2	1.0	1.3	–	1.3
OUES/kg	31.7 (10.1)	20.0	30.3	31.7	–	44.8
V_E/VCO_2 slope (units) (>31 abnormal)	34.8 (4.77)	41.7	31.8	34.4	–	31.4
% from expected value	12.6 (9.45)	25.6	3.0	10.0	–	12.0
$PetCO_2$ rest (mmHg) (<35 abnormal)	29.7 (4.27)	24	33	33	–	29
% from expected value	−15.1 (12.0)	−31.4	−6.0	−6.0	–	−17.1
O_2 pulse peak (ml/beat) (<14 abnormal)	6.25 (1.50)	7	4	7	–	7
% from expected value	−55.2 (10.5)	−50.0	−71.0	−50.0	–	−50.0
Laboratory data						
C-reactive protein (0.3–10 mg/L)	1.29 (2.00)	<0.30	0.42	<0.30	4.85	0.57
D-dimer (≤500 ng/ml)	623.5 (349 97592)	794	97.572	–	453	349
Fibrinogen (200–400 mg/dl)	289.2 (108.5)	311	190	217	465	263
Troponin (<0.004 ng/ml)	0.004 (0.001)	0.003	0.004	0.004	0.004	0.007

Abbreviations: BMI, body mass index; FMD, flow mediated dilatation; ICU, intensive care unit; LA, left atrium diastolic diameter; LVDD, left ventricle diastolic diameter; LVEF, left ventricle ejection fraction; LVPW, left ventricle posterior wall thickness; LVSD, left ventricle systolic diameter; MFR, myocardial flow reserve; MIS-C, multisystem inflammatory syndrome in children; OUES, oxygen uptake efficiency slope; Septum, interventricular septum thickness; V_E, pulmonary ventilation; VO_{2peak}, peak oxygen consumption; VO_{2VAT}, oxygen consumption at ventilatory anaerobic threshold.

[a]Patient 4 exhibited discrete pericardial effusion at. Categorical data were reported as percentages and continuous data as mean±standard deviation (SD) or median (range).

the blood pool and myocardial TACs, corrected for spill-over and partial volume. MFR was calculated as the ratio of stress MBF over the rest MBF (the 17-segment model according to the American Society of Nuclear Cardiology recommendations). For each left ventricle (LV) segment (see Figure 2), MFR at right coronary artery (RCA), left circumflex artery (LCX), left anterior descending (LAD) and MFR global were considered abnormal when <2, in gray zone when between 2 and 2.5 and normal when >2.5 (Duarte-Neto et al., 2021) (see Figures 1 and 2 for illustrative exams).

2.3 | Standard echocardiography

Standard transthoracic echocardiography was performed according to the recommendations of the American Society of Echocardiography (Farooqi et al., 2021).

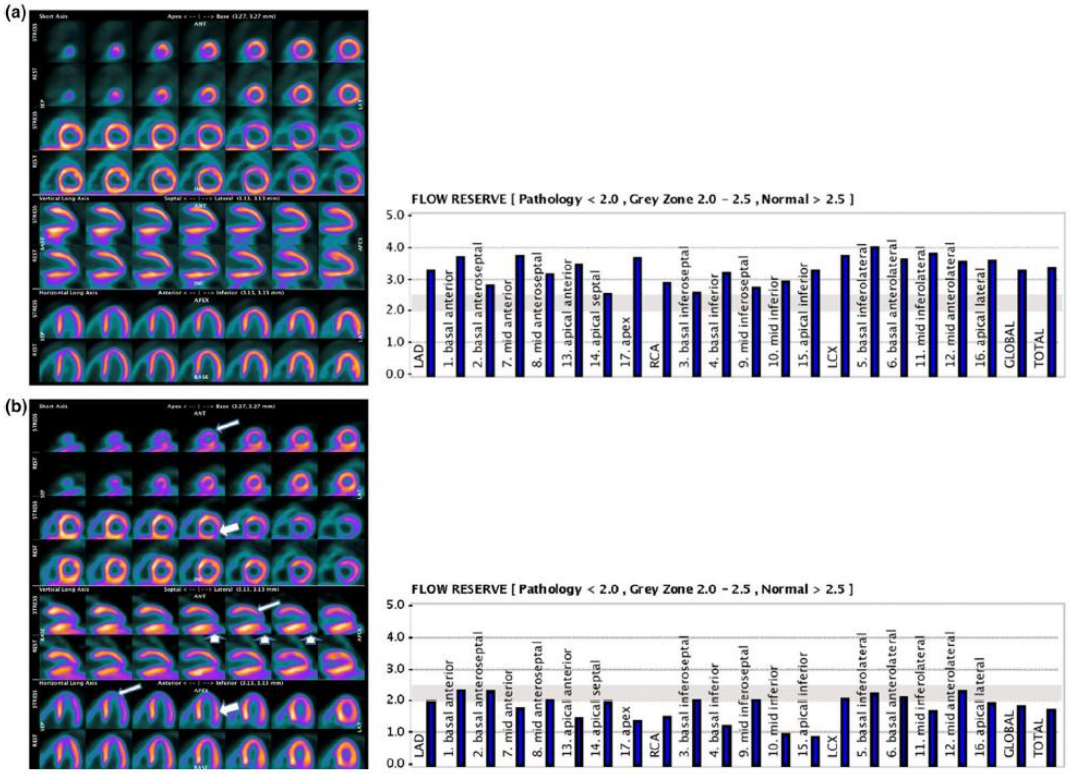


FIGURE 1 Illustrative data of ^{13}N-ammonia PET distribution: (a) Female patient, 9 years old, showed normal MFR. (b) Female patient, 8 years-old, showed abnormal values for MFR. Transient perfusion defects in the anteroapical (narrow arrows), inferoapical (thick arrows) and inferolateral (upright arrows) territories during the stress phase were observed. MFR, myocardial flow reserve

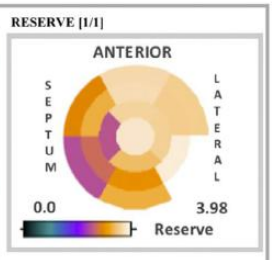
Cardiac chamber dimensions were obtained using two-dimensional mode and left ventricle ejection fraction (LVEF) was calculated by Simpson's method (normal LV EF ≥55%) (Farooqi et al., 2021). The *z*-score values of cardiac chambers were calculated according to Lopez et al (normal values between 2 and < +2.5). The equipment used was a Philips Affiniti 70, with multifrequency transducers (S 5-1 and S 8-3 MHz).

2.4 | Brachial FMD

FMD was evaluated according to current guidelines (Feldstein et al., 2020) using a high-resolution Doppler ultrasound machine (LOGIQ e PRO—GE Healthcare) equipped with a 4.0–12.0 MHz linear transducer. Initially, participants were positioned in the supine position with their right arm extended at an angle of ~80° from the torso. Longitudinal images of the brachial artery diameter were taken using the B-mode ultrasound, and simultaneous pulse-waved Doppler blood flow velocity was obtained using a 60° intonation angle with the sample volume placed in mid-artery and aligned with the blood flow. Initially, a 1-min baseline recording of the brachial artery diameter and blood flow velocity was performed. Then, the ischemic stimulus was performed by inflating a cuff placed in the forearm to 60 mmHg above the patient's resting systolic pressure for 5 min. Recordings were resumed 30 s before cuff deflation and continued for 3 min thereafter. Brachial artery diameter and shear rate (4 × mean blood velocity/internal diameter) were analyzed by a blinded evaluator using a semi-automatic edge-detection and wall-tracking software (Cardiovascular Suite, Quipu®). FMD was calculated as the percentage change of the brachial artery diameter after cuff release in relation to baseline brachial artery diameter [FMD = (baseline diameter−peak diameter baseline diameter) × 100]. To describe the relevant shear rate stimulus for FMD, we also calculated the area-under-the-curve of the shear rate up to the peak diameter (SRAUC). FMD lower than the

(a)

Parameter / Segment	F(STRESS) ml/min/g	relative [% of Max]	F(REST) ml/min/g	relative [% of Max]	CFR S/R
LAD	2.809		0.8588		3.271
1. basal anterior	2.9968	83.6	0.8125	78.8	3.687
2. basal anteroseptal	2.4662	68.8	0.8835	85.7	2.791
7. mid anterior	3.1435	87.7	0.8435	81.8	3.727
8. mid anteroseptal	3.1438	87.7	1.0071	97.7	3.122
13. apical anterior	2.6634	74.3	0.7701	74.7	3.458
14. apical septal	2.3215	64.8	0.9185	89.1	2.527
17. apex	2.7607	77.0	0.7615	73.9	3.625
RCA	2.7736		0.9658		2.872
3. basal inferoseptal	2.4164	67.4	0.9512	92.3	2.54
4. basal inferior	2.956	82.5	0.9316	90.4	3.173
9. mid inferoseptal	2.8105	78.4	1.0309	100.0	2.726
10. mid inferior	2.8512	79.5	0.982	95.3	2.903
15. apical inferior	2.989	83.4	0.9204	89.3	3.248
LCX	3.4281		0.9258		3.703
5. basal inferolateral	3.5839	100.0	0.9003	87.3	3.981
6. basal anterolateral	3.3109	92.4	0.9227	89.5	3.588
11. mid inferolateral	3.5019	97.7	0.9243	89.7	3.789
12. mid anterolateral	3.3898	94.6	0.9597	93.1	3.532
16. apical lateral	3.3093	92.3	0.9316	90.4	3.552
GLOBAL	2.9743		0.9103		3.267
TOTAL	2.9351	81.9	0.8836	85.7	3.322



(b)

Parameter / Segment	F(STRESS) ml/min/g	relative [% of Max]	F(REST) ml/min/g	relative [% of Max]	CFR S/R
LAD	2.3764		1.2274		1.936
1. basal anterior	2.8118	91.3	1.2125	90.0	2.319
2. basal anteroseptal	3.0813	100.0	1.3475	100.0	2.287
7. mid anterior	2.148	69.7	1.2202	90.5	1.76
8. mid anteroseptal	2.6695	86.6	1.3226	98.2	2.018
13. apical anterior	1.6389	53.2	1.1345	84.2	1.445
14. apical septal	2.3382	75.9	1.2059	89.5	1.939
17. apex	1.4887	48.3	1.0937	81.2	1.361
RCA	1.8981		1.2957		1.465
3. basal inferoseptal	2.5949	84.2	1.2928	95.9	2.007
4. basal inferior	1.5849	51.4	1.3064	96.9	1.213
9. mid inferoseptal	2.6197	85.0	1.3073	97.0	2.004
10. mid inferior	1.2043	39.1	1.2963	96.2	0.929
15. apical inferior	1.056	34.3	1.2625	93.7	0.836
LCX	2.6814		1.3087		2.049
5. basal inferolateral	2.9619	96.1	1.3385	99.3	2.213
6. basal anterolateral	2.8166	91.4	1.3432	99.7	2.097
11. mid inferolateral	2.1553	69.9	1.3058	96.9	1.651
12. mid anterolateral	3.0458	98.8	1.3262	98.4	2.297
16. apical lateral	2.2479	73.0	1.1847	87.9	1.897
GLOBAL	2.3133		1.2708		1.819
TOTAL	2.1462	69.7	1.2536	93.0	1.712

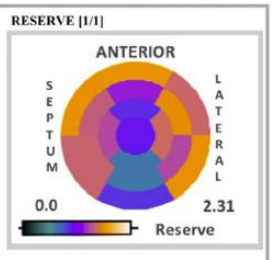


FIGURE 2 ^{13}N-ammonia PET data. Polar maps of MBF values with the table on 17 American Heart Association (AHA) segments. (a) Female patient, 9 years old, showed normal values for MFR. (b) Female patient, 8 years old, showed abnormal values for MFR. In the bullseye illustrations, white-to-purple means normal values of MFR and blue-to-black means abnormal or decreased flow reserve. MBF, myocardial blood flow; MFR, myocardial flow reserve

age- and sex-specific 25th percentile (Hadi et al., 2005) was considered as suggestive of endothelial dysfunction.

2.5 | Cardiopulmonary exercise test

A symptom-limited maximal cardiopulmonary exercise test was carried out on a treadmill (Centurion model 300; Micromed) using a ramp protocol test at a controlled room temperature (21–23°C). Peak oxygen consumption (VO_{2peak}), oxygen consumption at ventilatory anaerobic threshold (VO_{2VAT}), oxygen uptake efficiency slope (OUES), heart rate-oxygen consumption relationship (HR/VO_2 slope), oxygen pulse at peak of exercise (O_2 pulse peak), V_E/VCO_2 slope were measured breath-by-breath through a computerized system (MetaLyzer 3B; Cortex). One patient (P4) was prohibited to perform the test by the cardiologist because she had lower % LVEF and persistent discrete pericardial effusion. Reference values from healthy children sorted by age and sex were used for identifying abnormal exercise capacity (Holder et al., 2021; Hossri et al., 2019).

3 | RESULTS

The main findings can be seen in Table 1. P1 and P4 exhibited homogeneous rest but heterogeneous stress perfusion with perfusion defects developed in the slightly dilated left ventricular cavity, suggesting stress-induced

myocardial ischemia associated with MFR lower than 2.0 (LAD: 1.2; RCA: 2.0; and LCX: 2.1 and LAD: 1.9; RCA: 1.4; and LCX: 2.0) respectively, see P4 in Figures 1b and 2b.

All patients showed signs suggesting normal coronary arteries (all score-$z \geq 2.5$) (Farooqi et al., 2021) by standard echocardiogram at post-discharge, except for one patient (P4).

FMD assessment was not completed in one participant (P2) who experienced significant discomfort during the procedure. Of the remaining four participants, mean (SD) FMD% was 6.38 ± 3.41. Participants P1 and P4 presented with preserved FMD, while participants P3 and P5 had reduced FMD suggestive of endothelial dysfunction.

Mean (SD) VO_{2peak} was 26.3 ± 8.4 ml/kg/min. All patients showed abnormal VO_{2peak}, with lower predicted values (range: 35.2–64.5%). Similarly, all patients had lower predicted values for VO_{2VAT} (range: 15.6–38.2%), OUES (range: 1.0–1.3 L/min) and O_2 Pulse (range: 4–7 ml/beat). A ventilatory inefficiency was also identified, considering the mean (SD) value of V_E/VCO_2 Slope 34.8 ± 4.8 units (Hossri et al., 2019). Collectively, these findings indicate an impairment in cardiorespiratory and oxidative metabolism during physical exercise.

P1, P2, and P4 had abnormal values for D-dimer and fibrinogen, respectively. The other parameters were within normal range.

4 | DISCUSSION

This study reveals novel pathological findings in MIS-C patients which may help optimize treatment protocols in this condition. P1 and P4 exhibited impaired MFR, whereas P3 and P5 showed reduced endothelial function. All patients showed dysfunctional cardiorespiratory responses to a maximal exercise test.

To our knowledge, this is the first study to investigate myocardial perfusion and blood flow by PET imaging in a case series of MIS-C. This robust technique has been considered useful in clinical decision-making for patients with suspected coronary artery disease, as it can detect multivessel ischemia that could otherwise appears as normal on stress imaging if ischemia is global and balanced among all coronary territories (Lopez et al., 2010). The ratio of MBF at stress over rest is labeled MFR. It is primarily controlled by the release of local metabolites such as adenosine or nitric oxide. As the heart has minimal ability to increase oxygen extraction and rely on anaerobic metabolism, increased metabolic demands of the heart are met primarily via increases in coronary blood flow. In the absence of obstructive epicardial coronary artery disease, as it was the case of our patients presumably, coronary blood flow is primarily controlled by changes in resistance in the small arteries and arterioles (i.e., microvasculature), which play an important role in myocardial perfusion in general in regional and transmural distribution. Herein two patients showed abnormal MFR, which could be a consequence of coronary microvascular dysfunction, resulting from vasomotor dysregulation or endothelial dysfunction of the small coronary arterioles. In fact, our data add to post-mortem evidence suggesting that coronary microvascular involvement appears to comprise COVID-19/MIS-C pathophysiology (Penner et al., 2021). Of relevance, we also observed brachial endothelial dysfunction (as assessed by FMD) in two other patients different from those with abnormal findings upon PET imaging, suggesting that vascular involvement is not restricted to microvasculature in MIS-C. Collectively, the present results may be of clinical relevance since vascular dysfunction is a potentially reversible condition that is associated with future cardiovascular events (Singh et al., 2003).

Another striking finding was the abnormal cardiorespiratory response during exercise. Some metrics of impaired oxidative metabolism (e.g., lower VO_{2VAT} and OUES) and ventilatory inefficiency (e.g., higher VE/VCO_2 slope) were below normal values for all patients. Also, all patients showed lower $VO_{2peak,}$ which is an independent risk factor associated with poor prognosis in several diseases and all-cause mortality in general population (Thijssen et al., 2019). Rehabilitating cardiopulmonary capacity emerges as a potential therapeutic goal in MIS-C to prevent any cardiac events, improve patients' fitness and restore performance in daily living activities.

This study has limitations. First, given the paucity of ^{13}N-ammonia PET/CT studies and a large normal database in children, the arbitrary threshold limit (i.e., 2.5 ml/g/min) used to separate normal from abnormal MBF has not been yet validated in the pediatric population. Second, the low number of patients enrolled, and the lack of a control group without MIS-C and the longitudinal assessments preclude any causative inferences and insights on natural course of the syndrome. Therefore, studies assessing the frequency, predictors, clinical repercussion, and mechanisms of the cardiovascular and pulmonary findings described herein are warranted.

In conclusion, we reported on novel pathophysiological findings in MIS-C patients (i.e., reduced myocardial perfusion, cardiopulmonary capacity, and endothelial function), which advances the knowledge on this newly described condition and may help tailor better treatments for these patients. In-depth investigation using ^{13}N-ammonia PET-CT imaging, brachial FMD, and

cardiopulmonary exercise testing provides supplementary information that might be helpful in clinical decision-making in MIS-C care.

CONFLICT OF INTEREST

We declare no competing interests.

AUTHOR CONTRIBUTIONS

All the authors contributed substantially to the conception and design of the study and in the analysis and interpretation of data. All authors revised the work critically and approved the final version.

ETHICS APPROVAL STATEMENT AND CLINICAL TRIAL REGISTRATION

This study was approved local ethics committee (protocol #37460620.8.0000.0068) and registered at ClinicalTrials.gov (NCT04659486).

DATA AVAILABILITY STATEMENT

Access to de-identified data or related documents can be requested through the submission of a proposal with a valuable research question, necessary data protection plan, and ethical approvals. A contract will be signed. Data requests should be addressed to the corresponding author.

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4.3 Article 3: Home-Based Exercise Training in the Recovery of Multisystem Inflammatory Syndrome in Children: A Case Series Study

children

MDPI

Article

Home-Based Exercise Training in the Recovery of Multisystem Inflammatory Syndrome in Children: A Case Series Study

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Abstract: Objective: To assess the potential therapeutic role of exercise on health-related quality of life, assessed by the Pediatric Outcomes Data Collection Instrument (PODCI), coronary flow reserve (CFR), cardiac function, cardiorespiratory fitness, and inflammatory and cardiac blood markers in multisystemic inflammatory syndrome in children (MIS-C) patients. Methods: This is a case series study of a 12-wk, home-based exercise intervention in children and adolescents after MIS-C diagnosis. From 16 MIS-C patients followed at our clinic, 6 were included (age: 7–16 years; 3 females). Three of them withdrew before the intervention and served as controls. The primary outcome was health-related quality of life, assessed PODCI. Secondary outcomes were CFR assessed by 13N-ammonia PET-CT imaging, cardiac function by echocardiography, cardiorespiratory fitness, and inflammatory and cardiac blood markers. Results: In general, patients showed poor health-related quality of life, which seemed to be improved with exercise. Additionally, exercised patients showed improvements in coronary flow reserve, cardiac function, and aerobic conditioning. Non-exercised patients exhibited a slower pattern of recovery, particularly in relation to health-related quality of life and aerobic conditioning. Conclusions: Our results suggest that exercise may play a therapeutic role in the treatment of post-discharge MIS-C patients. As our design does not allow inferring causality, randomized controlled trials are necessary to confirm these preliminary findings.

Keywords: COVID-19; pediatric multisystem inflammatory disease; cardiovascular imaging; microvasculature; exercise training

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1. Introduction

Coronavirus disease 2019 (COVID-19) has impacted children and adolescents, with most cases being mild or asymptomatic [1]. However, multisystem inflammatory syndrome in children (MIS-C) may manifest as a rare complication 4 weeks after the SARS-CoV-2 infection, resulting in multiorgan system dysfunction, several clinical manifestations, and the need for pediatric intensive care unit (PICU) hospitalization [2–5].

The cardiovascular system is the most impacted one (80%), followed by hematologic (76%), mucocutaneous (74%), and respiratory (70%) systems [6]. The etiology of cardiovascular findings in MIS-C is likely multifactorial. The main cardiac manifestations involve arrhythmia, aneurysms, ventricular dysfunction, coronary artery dilation, conduction

abnormalities, and coronary microvasculature disease (CMD) [7–9]. Microvascular dysfunction is considered the main driver of morbidity and mortality in COVID-19, and the severity of CMD associates with inflammatory markers, fibrin turnover, myocardial injury, and myocyte stretch [8].

Recently, we observed reduced coronary flow reserve (CFR) and impaired cardiopulmonary exercise capacity in a small cohort of MIS-C survivors [10]. CFR is the measure of the microvasculature's ability to respond to a stimulus, and as such, a proxy of small vessel function [11]. Reduced CFR may predict CMD, myocardial ischemia, arrhythmia, and sudden death during strenuous activity [12]. Low aerobic fitness is a determinant of premature death and is implicated in several comorbidities, including physical dysfunction, exercise intolerance, and chronic fatigue. Given the systemic and debilitating nature of MIS-C, one could expect that this condition could also impair, to some extent, overall health-related quality of life [13].

To date, there is limited evidence on the management of MIS-C complications. In this scenario, exercise emerges as a safe and non-expensive tool that could be useful to rehabilitate MIS-C patients, potentially offsetting persistent impairments in cardiovascular function and physical capacity, ultimately resulting in better health-related quality of life. Physical exercise promotes an anti-inflammatory response and improves immune defense through the involvement of diverse organs (e.g., heart, lungs, skeletal muscle, brain, and intestines). The anti-inflammatory response to exercise has also been associated with a reduced risk of developing comorbidities (such as obesity) and reduced cardiovascular morbidity [14,15]. This hypothesis is supported by analogy, considering the beneficial effects of exercise in other pediatric acute or chronic conditions [16–18].

In this case series study, we aimed to explore the potential therapeutic role of exercise on several outcomes (i.e., health-related quality of life, CFR, cardiac function, cardiorespiratory fitness, and inflammatory and cardiac blood markers) in MIS-C patients.

2. Material and Methods

2.1. Study Design and Patients

This is a case series study of a 12-wk home-based exercise intervention in children and adolescents who survived MIS-C. This study is part of a prospective cohort study aimed to explore the long-term effects of SARS-CoV-2 infection in surviving pediatric post-COVID-19 and MIS-C patients (clinicaltrials.gov NCT04659486). Data from the patient's acute phase MIS-C were retrospectively assessed through medical records. The post-infection data were collected prospectively in an outpatient clinic for COVID-19 at the Children's and Adolescents' Institute of the Clinical Hospital of the University of Sao Paulo between October 2020 and January 2022. Out of the 16 MIS-C patients followed at our clinic, 4 died, 3 did not meet inclusion criteria (i.e., younger than 7 years), and 3 did not accept to participate in this exercise trial. Therefore, 6 patients were included. All patients (age: 7–16 years; 3 females) fulfilled the MIS-C diagnosis according to the Center for Disease Control (CDC) criteria [19]. Five patients had positive serologic tests (assessed by real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) or antibody testing. Real-time RT-PCR to evaluate SARSCoV-2 RNA was performed on swab-collected nasopharyngeal and/or oropharyngeal samples [20]), and 1 had a negative serologic test but was exposed to a confirmed COVID-19 case within 4 weeks prior to the onset of symptoms. Demographic and clinical data at hospital admission can be seen in Table 1. Five out of six patients were admitted to PICU. Three patients required respiratory support and oxygen therapy, and three had a vasodilatory shock. The median length of stay was 11 (range: 5–18) days. The median time elapsed from discharge to the beginning of the exercise training was 5.8 (range: 1.5–10) months. Of these 6 patients who initially accepted to participate in the trial, 3 of them gave up before the intervention. We decided to follow-up on them with all the planned assessments as we found it informative to have some sort of "control data" (from non-exercised patients), despite the non-random feature of this design.

Table 1. Clinical features among MIS-C patients during the acute phase.

Patient's Characteristics	Exercised Patients			Non-Exercised Patients		
	PI	**PII**	**PIII**	**PIV**	**PV**	**PVI**
Sex	Female	Male	Female	Female	Male	Male
Age (years)	16	7	9	8	11	11
Previously medical history	No	No	No	No	Yes (T1D)	No
Height (cm)	156	126	145	135	139	138
Weight (kg)	60.3	33.7	38.6	38.4	31.5	29.3
BMI ($kg \cdot m^{-2}$)	24.7	21.2	18.3	21.1	16.3	15.3
Adherence to 12-wk home-based exercise program (supervised/online)	83.3/62.5	100/100	100/37.5	*	*	*
	Signs and symptoms at admission					
Fever (days)	Yes (12)	Yes (8)	Yes (12)	Yes (1)	Yes (3)	Yes (5)
Conjunctivitis	Yes	Yes	No	No	Yes	Yes
Arterial hypotension	Yes	Yes	Yes	No	Yes	Yes
Shock	No	Yes	Yes	No	Yes	Yes
Abdominal pain	Yes	Yes	Yes	Yes	Yes	Yes
Diarrhea	Yes	No	No	Yes	No	Yes
Echocardiogram abnormalities	Yes *	Yes #	Yes *	No	Yes $	No
	Treatment					
ICU admission	No	Yes	Yes	Yes	Yes	Yes
Length of stay at hospital (days)	3	14	18	5	10	12
Respiratory support/Oxygen therapy	No/No	Yes/Yes	No/No	No/No	Yes/No	Yes/Yes
Anti-inflammatory treatment	No	Yes (mPRED)	Yes (mPRED)	No	Yes (mPRED)	No
Immunoglobulin treatment	First dose 2 g/kg	First and second dose 2 g/kg	First dose 2 g/kg	First dose 2 g/kg	First dose 2 g/kg; second dose 1 g/kg	First dose 2 g/kg

Abbreviation: T1D: type 1 diabetes; BMI: body mass index; ICU: intensive care unit; mPRED: Methylprednisolone. * Pericardial effusion; # Echogenicity of coronary arteries, without dilation; $ Mild ectasia of right and left coronary arteries.

The primary outcome was health-related quality of life, assessed by the Pediatric Outcomes Data Collection Instrument (PODCI). Secondary outcomes were CFR assessed by 13N-ammonia PET-CT imaging, cardiac function by standard echocardiography, cardiorespiratory fitness, and inflammatory and cardiac blood markers (C-reactive protein, D-dimer, fibrinogen, troponin T, hemoglobin, lymphocyte and platelet count, urea, creatinine, alanine, and aspartate transaminase).

2.2. Ethics

The protocol was approved by the National and Institutional Ethical Committee of Clinical Hospital, CAAE: 37460620.8.0000.0068. Patients and guardians signed an informed consent before participants' enrollment, and the study was conducted according to the Declaration of Helsinki.

2.3. Exercise Training Program

The 12-week, 3-times-a-week, home-based exercise program used herein was thoroughly described elsewhere [21,22]. In brief, the bouts had two components. The first one included aerobic exercise predominantly, such as jumping jacks, skipping, flexibility, and mobility exercises. The second component included bodyweight exercises (push-ups, air squats, lunges, crunches, and planks). Exercise sessions occurred 3 times a week; one weekly session was supervised online by a fitness trainer, whereas the other two were unsupervised, with patients being advised to provide feedback to the staff upon the completion of the training bout. The training monitoring was conducted through WhatsApp®, Zoom®, or Google Meets® apps. The exercise intensity was assessed through the Children's OMNI scale of perceived subject exertion (PSE) [23]. Progression was carried out every

4 weeks using the OMNI scale (week 1–4, PSE: 5–6; week 4–8, PSE: 6–7 and week 8–12, PSE: 7–8), modifying the number of repetitions (10 to 15), sets (3 to 4), and/or duration of the sets (30 to 45 s). Patients received instructional videos, photos, and "gifs" describing the exercise program. A video call was conducted prior to program initiation to provide details on the program and collect information on the patient's health status. We assessed adherence to the intervention using a training log.

2.4. Pediatric Outcomes Data Collection Instrument (PODCI)

To assess health-related quality of life, we used PODCI, which evaluates functional health status through an 83-86 item questionnaire [24]. This questionnaire consists of scores: four encompassing physical function (upper extremity and physical functioning, transfer and basic mobility, sports, and physical functioning) and two assessing psychological well-being (pain/comfort and happiness), and a PODCI global function score (0–100). Lower scores are indicative of a lower health-related quality of life. A radar plot was created using absolute values from pre and post-periods for each patient and each PODCI domain (upper extremity and physical functioning, transfer and basic mobility, sports and physical function, pain/comfort, and happiness). All radar plots were generated using the *fmsb* package (v. 0.7.5; Nakazawa, M., 2023) in the environment R (version 3.5.3; R Core Team 2020).

2.5. Coronary Flow Reserve Imaging Protocol by 13N-Ammonia PET-CT (13N PET-CT)

Cardiac positron emission tomography-computed tomography (PET-CT) is the gold standard noninvasive test for myocardial blood flow (MBF). The ratio of the MBF measured at maximal vasodilation over the MBF at rest is referred to as myocardial flow reserve (MFR) or coronary flow reserve (CFR), which is a measure of the vasodilatory reserve of the myocardium. Reduced CFR, in the absence of flow-limiting coronary artery disease, is believed to reflect dysfunction in the myocardial microvasculature and result in ischemia [11,25]. Protocol details can be seen in our previous work [10].

CFR was calculated as the ratio of stress MBF over the rest MBF (for each left ventricle (LV) segment (see Table 2), CFR at right coronary artery (RCA), left circumflex artery (LCX), left anterior descending (LAD), and the 17-segment model according to the American Society of Nuclear Cardiology recommendations [26] were considered abnormal when <2, borderline between 2 and 2.5, and normal when >2.5 [27].

Standard transthoracic echocardiography was performed according to the recommendations of the American Society of Echocardiography and included M-mode, two-dimensional imaging, conventional, and tissue Doppler evaluation at the septal and lateral mitral annulus [28]. The equipment used was a Philips Affiniti 70 (Andover, MA 01810 USA), with multifrequency transducers (S 5–1 and S 8–3 MHz). Cardiac chamber dimensions were obtained using two-dimensional mode, and left ventricle ejection fraction (LVEF) was calculated by Simpson's method (normal LVEF $\geq$ 55%) [28].

The z-score values for the measures were calculated according to the "Boston Children's Hospital z-score system" [29]. LV mass (g) was estimated using Devereaux's formula according to the Penn convention and indexed for height (m) raised to an exponential power of 2.7; we used Omni calculator [28]. LV hypertrophy was diagnosed whenever the LV mass index was greater than the 95th percentile for sex and age, according to Khoury et al. [30]. The evaluation of LV diastolic function included mitral E/e' ratio, with e' being the average of values obtained by tissue Doppler at the septal and lateral annulus (normal E/e' < 14) [28]. Right ventricular (RV) systolic function was assessed by tricuspid annular plane systolic excursion (TAPSE). RV systolic dysfunction was detected when the TAPSE z-score was less than −2 [31]. E and A wave velocities, E/A wave ratio, DT, and E/E' ratio (diastolic filling pressure surrogate) will also be measured. Diastolic dysfunction was classified as mild, moderate, or severe according to current guidelines [32]. Diastolic function will be considered impaired if there is evidence of left atrial enlargement or abnormal filling pressure.

Table 2. Distribution of LV segments according to 13N PET-CT CFR categories for each MIS-C patient pre and post-12-wk exercise training program.

		Exercised Patients		
		Pre	Post	Fisher's Test
I	CFR abnormal	12/20	2/20	0.0022
	CFR borderline	5/20	6/20	1.0000
	CFR normal	3/20	12/20	0.0079
II	CFR abnormal	0/20	0/20	1.0000
	CFR borderline	1/20	6/20	0.0915
	CFR normal	19/20	14/20	0.0915
III *	CFR abnormal	11/20	-	-
	CFR borderline	9/20	-	-
	CFR normal	0/20	-	-
		Non-exercised patients		
IV	CFR abnormal	0/20	6/20	0.0202
	CFR borderline	0/20	6/20	0.0202
	CFR normal	20/20	8/20	0.0001
V	CFR abnormal	1/20	1/20	1.0000
	CFR borderline	1/20	6/20	0.0915
	CFR normal	18/20	13/20	0.1274
VI	CFR abnormal	0/20	8/20	0.0033
	CFR borderline	9/20	12/20	0.0012
	CFR normal	11/20	0/20	0.0001

Abbreviations: LV: left ventricle; CFR: coronary flow reserve. * PIII did not complete the post-test PET-CT exam due to personal reasons.

2.6. 2 DST Echocardiography and Speckle Tracking

The main principle of 2DST is that each segment of myocardial tissue displays a specific pattern of gray values in the ultrasound image, commonly referred to as a speckle pattern. Tracking this acoustic pattern during the cardiac cycle enables the observer to follow the myocardial motion and to directly assess ventricular deformation [33]. To evaluate segmental LV longitudinal systolic strain, two-dimensional harmonic image cine-loop recordings of apical four-, three-, and two-chamber views were acquired and stored digitally for analysis. A sector scan angle of 30–60° and frame rates of 60–90 Hz were chosen. A good-quality electrocardiogram signal was obtained simultaneously. The endocardial and epicardial tracing was automatically generated by the computer algorithm and manually adjusted to cover the whole myocardium wall, when necessary (QLabTM software, Philips) [33]. The extent of myocardial strain in the longitudinal direction throughout the cardiac cycle was computed as percentages (absolute values), represented by the global longitudinal strain (GLS%).

2.7. Cardiopulmonary Exercise Test

A symptom-limited maximal cardiopulmonary exercise test was carried out on treadmill. CPETs were performed on a treadmill with an intensity-graded, maximal effort protocol and continuous gas exchange (Metalyzer IIIb/breath-by-breath). All tests in this study were performed by the same intrahospital laboratory at controlled room temperature with individuals in an upright position (20–23 °C). Test termination was determined by volitional exhaustion, and maximal effort was confirmed by a peak respiratory exchange ratio > 1.10, maximal heart rate > 95% age/gender-predicted values, or maximum rating of perceived exertion (RPE) [34]. The outcomes were absolute, and % predicted values of oxygen consumption at ventilatory anaerobic threshold (VO_{2VAT}) (mL/kg/min) and VO_{2peak} (mL/kg/min). Twelve-lead ECG and gas exchange measurements were recorded continuously. Peak VO_2 was determined as the mean value of VO_2 during the final 30 s of the graded exercise test. The following variables were obtained breath-by-breath and expressed as 30 s averages: pulmonary oxygen uptake (VO_2 $mL \cdot kg^{-1} \cdot min^{-1}$ standard temperature and pressure, dry); pulmonary ventilation (V_E; L/min body temperature

and pressure, saturated); end-tidal carbon dioxide pressure ($PetCO_2$; mmHg), ventilatory equivalent ratio for carbon dioxide (V_E/VCO_2), and ventilatory equivalent ratio for oxygen (V_E/VO_2).

Other CPET variables such as the peak oxygen consumption (VO_{2peak}), oxygen consumption at the ventilatory anaerobic threshold (VO_{2VAT}), V_E/VCO_2 slope, the lowest V_E/VCO_2 and respiratory exchange ratio (RER), chronotropic reserve (CR) and heart rate peak (HRP) were analyzed as previously described [34]. For all dependent variables, reference values from healthy children sorted by age and sex groups, whenever available, were used for identifying a normal exercise capacity [35,36].

2.8. Statistical Analysis

Categorical data were reported as percentages and continuous data as mean ± standard deviation (SD). Fisher's exact test was used to compare the frequency of coronary flow reserve (abnormal < 2, borderline between 2 and 2.5, and normal when >2.5), as seen in Table 2. The significance level was set at $p \leq 0.05$.

3. Results

3.1. Exercised Patients

Figure 1 displays the flowchart of patients followed in our tertiary hospital.

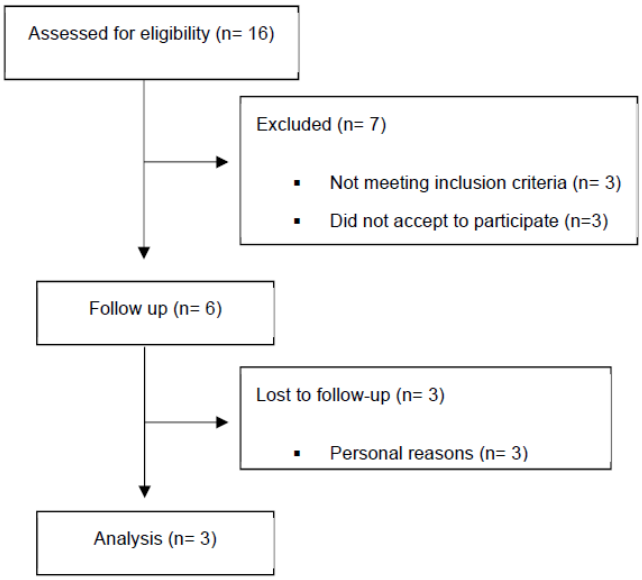

Figure 1. Flowchart of MIS-C patients from our tertiary hospital.

Patient I is a 16-year-old female who presented with fever, conjunctivitis, arterial hypotension, abdominal pain, and diarrhea, with no need for ICU admission (Table 1). The patient attended 83% of the supervised exercise sessions and 62% of the online sessions. After the intervention period, the global PODCI score improved by 18% (Figure 2), with the greatest improvements being seen in the pain score (194%). After exercise, abnormal CFR reduced from 12/20 to 2/20 segments, $p = 0.0222$ (Table 2), whereas normal CFR significantly increased from 23/20 to 12/20, $p = 0.0079$. LVEF% improved from 56% to 64% (Figure 3).

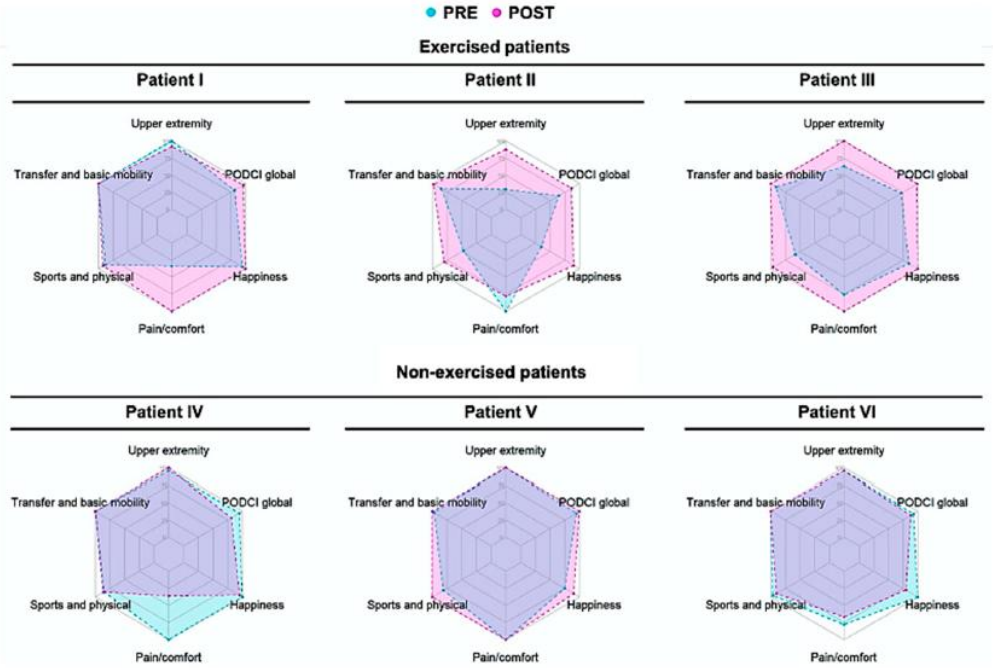

Figure 2. Radar-plot: Healthy-related quality of life domains in exercised and non-exercised MIS-C patients.

Noticeably, the time to exhaustion and VO_{2peak} increased by 22% and 14%. In addition, the chronotropic reserve increased by 21% and HRP by 10% (Table 3). D-dimers reduced by 16%, whereas the other laboratory markers were within the normal range throughout the intervention.

Table 3. Cardiopulmonary exercise test and laboratory findings among MIS-C patients pre and post-12-wk home-based program.

	Exercised Patients			Non-Exercised Patients		
	PI	PII	PIII	PIV	PV	PVI
Cardiopulmonary exercise test						
Time to exhaustion (min)						
Pre	9.0	7	9.2	11.3	11.4	10
Post	11.0	10.5	8.5	9.3	12.3	10
Δ, %	22.2	50.0	−7.61	−17.7	7.89	0
VO_{2peak} ($mL \cdot kg^{-1} \cdot min^{-1}$)						
Pre	22.5	18.3	30.8	35.2	42.5	37.1
Post	25.6	20.7	29.3	28.4	48.9	38.7
Δ, %	13.8	13.4	−4.87	−19.2	15	4.39
VO_{2VAT} ($mL \cdot kg^{-1} \cdot min^{-1}$)						
Pre	9.66	12.9	11.1	14.6	16.2	19.2
Post	9.73	15.6	15.8	12.9	24.8	16.7
Δ, %	0.72	20.9	−29.6	−11.8	52.8	−13.1
% Predicted VO_{2peak} (<80% abnormal)						
Pre	48.7	37.1	70.1	64.5	78.7	69.4
Post	55.5	42.1	66.7	62.8	90.5	72.5
Δ, %	13.8	13.4	−4.87	−2.54	14.9	4.39
V_E/VCO_2 slope (units) (>31 abnormal)						
Pre	38.8	31.6	33.6	33.0	39.5	31.3
Post	42.2	32.8	36.9	36.1	39.6	37.8

Table 3. *Cont.*

	Exercised Patients			Non-Exercised Patients		
	PI	**PII**	**PIII**	**PIV**	**PV**	**PVI**
Δ, %	8.76	3.80	9.82	9.39	0.25	20.7
$PetCO_2$ rest (mmHg) (<35 abnormal)						
Pre	24	32	31	35	37	35
Post	27	29	30	35	36	30
Δ, %	12.5	−9.38	−5.88	0	−2.7	−14.3
O_2 pulse peak (mL/beat) (<14 abnormal)						
Pre	8	4	7	10	7	7
Post	10	5	7	8	9	7
Δ, %	25	25	0	−20	28.5	0
Resting heart rate (beats/min)						
Pre	91	107	120	93	100	115
Post	94	99	114	94	91	90
Δ, %	9.5	−7.4	−5	1.1	−9.0	−21.7
Heart rate peak (beats/min)						
Pre	178	134	195	188	195	170
Post	195	139	183	208	195	171
Δ, %	9.5	3.7	−6.1	10.6	0	0.6
Chronotropic reserve (%) (abnormal < 80%)						
Pre	87.9	35.5	112	94.6	106.7	75.3
Post	106.3	47.6	94.5	118.8	106.1	82.7
Δ, %	20.9	34.0	−15.5	25.5	−0.58	9.70
Laboratory data (normal range)						
C-reactive protein (0.3–10 mg/L)						
Pre	0.30	0.42	4.85	0.30	0.30	0.57
Post	0.71	1.06	2.90	0.32	0.60	0.30
Δ, %	136.6	152.3	−40.2	6.67	100	−47.3
D-dimers (≤500 ng/mL)						
Pre	794	97572	1691	*	271	349
Post	665	215	640	*	326	337
Δ, %	−16.2	−9978	−62.1	*	20.3	−3.44
Fibrinogen (200–400 mg/dL)						
Pre	311	190	465	*	304	263
Post	349	315	340	239	257	252
Δ, %	12.2	65.8	−26.8	*	−15.4	−4.18
Troponin T (<0.004 ng/mL)						
Pre	0.005	0.004	0.004	*	0.003	0.007
Post	0.003	0.004	0.003	*	0.003	0.003
Δ, %	−40.0	0	−25.0	*	0	−57.1
Hemoglobin (11.5–15.5 g/dL)						
Pre	10.9	14.6	12.7	12.7	12.3	11.2
Post	10.8	13.7	13.4	12.8	12.2	12.3
Δ, %	−0.92	−6.16	5.51	0.79	−0.81	9.82
Lymphocyte count ($1.5^{-7} \times 10^9$/L)						
Pre	3.29	6.29	3.51	2.90	1.72	2.10
Post	2.40	5.18	2.53	2.56	1.55	2.28
Δ, %	−27.0	−17.6	−27.9	−11.7	−9.88	9.09
Platelet count ($150^{-400} \times 10^9$/L)						
Pre	413	473	409	336	194	376
Post	368	359	405	314	178	449
Δ, %	−10.9	−24.1	−0.98	−6.55	−8.25	19.4
Urea (7–20 mg/dL)						
Pre	23	27	18	18	23	41
Post	27	24	12	17	23	24
Δ, %	17.3	−11.1	−33.3	−5.56	0	−41.6
Creatinine (0.59–1.53 mg/dL)						
Pre	0.61	0.47	0.46	0.46	0.48	0.44
Post	0.77	0.54	0.54	0.50	0.53	0.52
Δ, %	26.2	14.9	17.3	8.70	10.4	18.1

Table 3. *Cont.*

	Exercised Patients			Non-Exercised Patients		
	PI	**PII**	**PIII**	**PIV**	**PV**	**PVI**
Alanine transaminase (7–55 U/L)						
Pre	20	19	36	20	19	25
Post	15	19	26	15	13	23
Δ, %	−25.0	0	−27.7	−25.0	−31.6	−8.0
Aspartate transaminase (8–33 U/L)						
Pre	13	11	26	12	9	12
Post	9	12	19	11	7	10
Δ, %	−30.7	9.09	−26.9	−8.33	−22.2	−16.6

Abbreviations: VO_{2peak}: peak oxygen consumption; VO_{2VAT}: oxygen consumption at ventilatory anaerobic threshold; V_E/VCO_2: PetCO2; Δ = Delta.

Patient II is a 7-year-old male who presented with fever, conjunctivitis, arterial hypotension, shock, and abdominal pain, requiring PICU admission, respiratory support, and oxygen therapy (Table 1). The patient completed 100% of both supervised and online exercise sessions. After the intervention period, the global PODCI score improved by 32% (Figure 1), with greater improvements seen in the upper extremity and happiness scores (203% and 157%).

Despite the severity of the case, following hospital discharge, CFR values were all $p > 0.05$ (Table 2), and cardiac function were normal on the interim visits with no change in medication (acetylsalicylic acid 100 mg/day). The time to exhaustion and VO_{2peak} increased by 50% and 13%. In addition, the chronotropic reserve increased by 34% and the HRP by 4% (Table 3). Inflammatory markers and other laboratory findings remained within the normal range throughout the follow-up period; however, d-dimers values dramatically reduced (9978%), stabilizing at the normal range.

Patient III is a 9-year-old female who presented with fever, arterial hypotension, shock, and abdominal pain, with a need for ICU admission (Table 1). The patient compliance to the intervention was 100% to the supervised sessions, but only 37% to the online exercise sessions. After the intervention period, the global PODCI score improved by 37% (Figure 1), with greater values seen for upper extremity and sports score delta (59% and 65%).

Before exercise, 13N PET-CT exam showed homogeneous rest but heterogeneous stress perfusion with perfusion defects developed in the slightly dilated left ventricular cavity, suggesting stress-induced myocardial ischemia associated with CFR < 2.0 in 57% and borderline 43%, with no normal values. PIII, unfortunately, did not attend the post-intervention 13N PET-CT exam due to personal reasons.

At baseline, 2DST echocardiography showed a slight alteration in TAPSE (−2.07) and reduced LVEF (54%), and the speckle tracking exam showed reduced GLS (−17.4%). Despite after exercise, the standard echocardiography showed normal values for cardiac function, including GLS% (Figure 3), the speckle tracking exam showed an impairment of the strain in the basal and middle segments of the infero-septal and inferior walls of the left ventricle (−13%). PIII had impaired time to exhaustion and VO_{2peak} (−7% and −5%). In addition, the chronotropic reserve reduced −15% and HRP −6% (Table 3). Nonetheless, inflammatory and cardiac markers improved (i.e., CRP, d-dimers, fibrinogen, and troponin T), whereas the other laboratory markers remained unaltered.

3.2. Non-Exercised Patients

Patient IV is an 8-year-old female who presented with fever, abdominal pain, diarrhea, and no echocardiography abnormalities at the acute phase (Table 1). At the final evaluation, the Global PODCI score was reduced −15% (Figure 1), with the greatest reduction being in pain score (−64%). At baseline, all CFR values were normal. At the final evaluation, 6/20 CFR values were abnormal ($p = 0.0202$), 6/20 borderline ($p = 0.0202$), and 8/20 within normal values ($p = 0.0001$) (Table 2). Additionally, cardiorespiratory fitness (e.g., time

to exhaustion and VO_{2peak}) reduced by 18% and 19% throughout the follow-up period. Cardiac function and laboratory markers remained within normal values (Table 3).

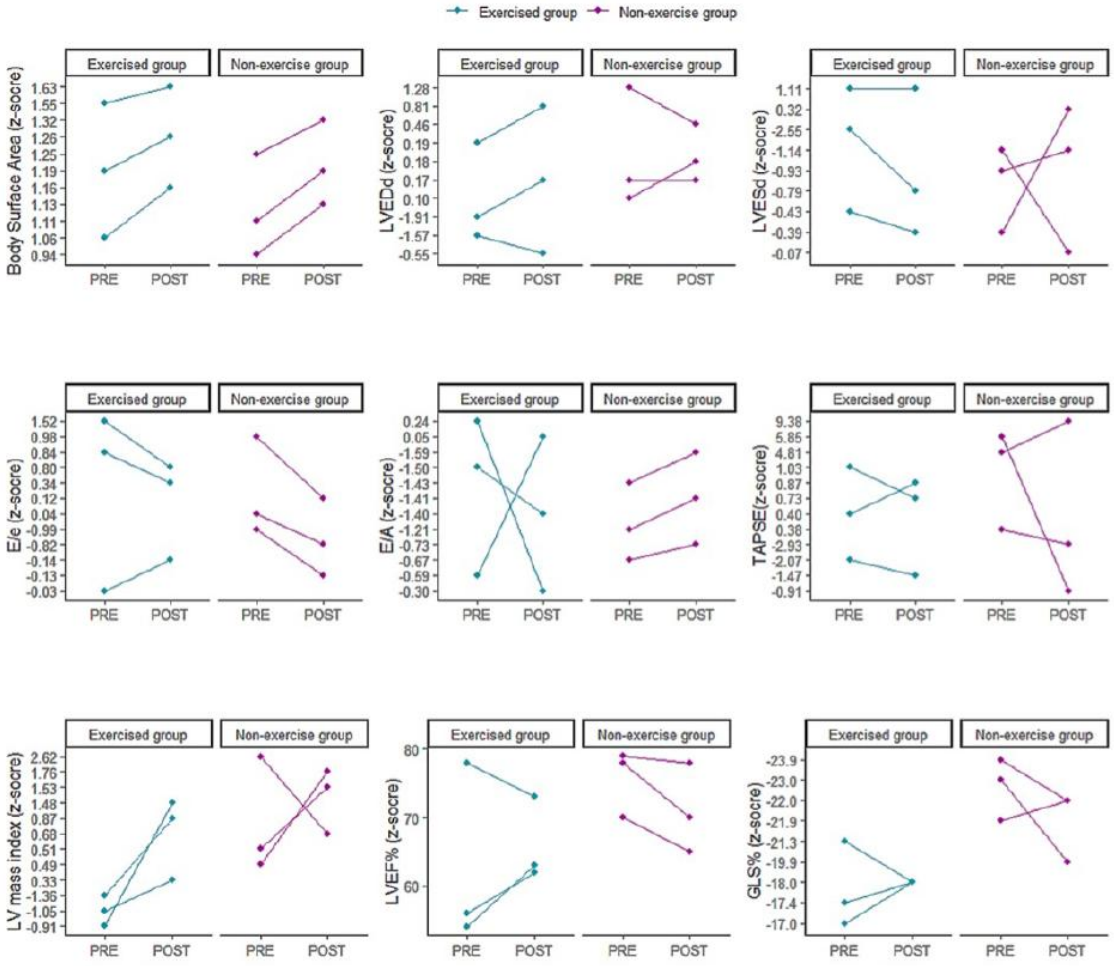

Figure 3. Echocardiographic parameters responses after exercise training in MIS-C patients. Abbreviations: LVEDd, left ventricle end-diastolic dimension; LVESd, left ventricle end-systolic dimension, E/A, early to late diastolic flow velocity ratio; TAPSE, tricuspid annular plane systolic excursion; LVEF, left ventricular ejection fraction; GLS, global longitudinal strain.

Patient V is an 11-year-old male with type I diabetes who presented with fever, conjunctivitis, hypotension, shock, and abdominal pain. He needed ICU admission and respiratory support, and echocardiography abnormalities were detected at the acute phase (Table 1). At the final evaluation, the Global PODCI score improved by 5% (Figure 1), whereas the others score did not change. Abnormal CFR values did not change, and borderline values raised from 1/20 to 6/20 ($p = 0.0915$), whereas normal values reduced from 18/20 to 13/20 ($p = 0.1274$). Time to exhaustion and VO_{2peak} values improved by 8% and 15%, respectively. Cardiac function and laboratory markers remained in the normal range.

Patient VI is an 11-year-old male who presented with fever, conjunctivitis, arterial hypotension, shock, abdominal pain, and diarrhea. He needed ICU admission and respiratory and oxygen support, but no echocardiography abnormalities were seen in the acute

phase (Table 1). At baseline, the 2DST echocardiography and speckle tracking showed normal cardiac function; however, at the final evaluation, the 2DST echocardiography showed normal values for cardiac function, including GLS (19.9%), whereas the speckle tracking exam showed impairment in the strain at the basal segment of the anteroseptal wall was −12% and the anterior wall −15%. The strain in the mid-segment of the inferoseptal and inferolateral walls were −15% and −16%. In line with these findings, abnormal CFR s increased from 0 to 8/20 ($p = 0.0033$), borderline significantly increased from 9/20 to 12/20 ($p = 0.0012$), and normal values were reduced from 11/20 to 0/20 ($p = 0.0001$). Cardiorespiratory fitness and laboratory markers remained unchanged.

4. Discussion

To our knowledge, this was the first study to demonstrate the effects of an exercise training program on health-related quality of life, microvascular and cardiac function, cardiorespiratory fitness, and inflammatory and cardiac blood markers in MIS-C survivors. To date, our findings suggest that exercise may positively impact the health-related quality of life, coronary flow reserve, cardiac function, and aerobic conditioning.

Pediatric-COVID patients have shown reduced health-related quality of life and at least one symptom related to long-COVID 4 months after hospital discharge [37]. Moreover, biopsychosocial stressors imposed by the pandemic may increase the signs and symptoms such as anxiety, depression, weight gain, physical inactivity, and possibly increase cardiovascular risk [38,39]. In this scenario, exercise training emerges as a potential therapeutic tool to mitigate the impaired health-related quality of life and other possible biopsychosocial side effects of the COVID-19 pandemic period in children's and adolescents' health.

Overall, patients assessed herein, particularly those included in the exercised group, showed poor health-related quality of life. However, exercise training programs lead to an improvement in these parameters for all patients, indicating a key role of exercise in the management of MIS-C patients. Moreover, patients who followed the standard of care did not have low quality-of-life scores in the initial assessment, preventing significant improvements in the domains assessed through the PODCI.

Cardiovascular complications arising from acute COVID-19 have been shown in the literature [40], inclusive in pediatric patients [38,41], which may increase the risk of cardiac events. In this scenario, the safety and efficacy of exercise training programs should be considered. In the current study, no mild, moderate, or severe side effects related to home-based exercise training program was reported during supervised or online sessions. Furthermore, it is noteworthy that exercised patients demonstrated an improvement in the microvascular and cardiac function (CFR, LVEF%), except for patient III, that did not perform the post-test PET-CT exam and showed defects in the segmental cardiac strain at post-test. In contrast, all non-exercised patients showed a higher frequency of abnormal CFR segments, indicating the absence of improvements in microvascular and cardiac function. Our hypothesis is that the adherence of PIII (37.5%) to supervised sessions was low, and the possible cardiovascular effects and adaptations related to physical exercise were not achieved. These findings are quite relevant since the home-based exercise training program showed to be safe and efficient in improving cardiovascular and cardiac function, lowering the possible cardiovascular risk in post-discharged MIS-C patients.

Some studies have reported impaired microvasculature in COVID-19 and MIS-C patients [42]. The mechanisms underlining the pathophysiology of disease are not fully elucidated. Myocardial ischemia and, hence, angina result from excessive myocardial oxygen that overcomes the oxygen supply. In some cases, transient ischemia can yield to persistent dysfunction following the restoration of flow. It is also possible that persistent asymptomatic ischemia leads to LV dysfunction, which mimics nonischemic causes of heart failure [43].

Blomster et al. analyzed the correlation between CFR, exercise capacity, and cardiac systolic and diastolic function in 400 adult patients with coronary artery disease (CAD). The authors showed that maximal exercise capacity is dependent on coronary CFR in

non-obstructive CAD, emphasizing the significance of microvascular circulation on cardiac performance [44].

The abnormal CFR detected by PET-CT in our surviving MIS-C patients (PIII and PVI), mirrored by segmental peak systolic longitudinal strain reduction, seems to indicate residual damage in the coronary microcirculation as a consequence of infection. Importantly, microcirculatory impairment (segmental strain reduction) was detected at the standard echocardiography even in the absence of coronary artery aneurysm.

13N PET-CT-measured impaired vasodilation capacity is associated with the increased risk of progression of congestive heart failure and mortality in adults with idiopathic cardiomyopathy [45]. Therefore, the long-term impacts of myocardial microvasculature compromise in MIS-C patients should be investigated.

The strengths of this study involved the investigation of patients with a rare condition and the use of gold standard methods to assess cardiac-and fitness outcomes, as well as the implementation of a newly developed home-based exercise training program aimed at treating MIS-C patients. The main limitations included the low number of patients enrolled (owing to the rarity of the condition) and the lack of a control group without MIS-C, which hampers establishing causation and limits possible insights on the natural course of the syndrome.

To conclude, our results may suggest that exercise could be a useful tool in the management of MIS-C patients. Randomized controlled studies are needed to confirm or refute these exploratory findings.

Author Contributions: Conceptualization, C.A., M.F.B.P., H.H.M., C.A.B., C.A.S. and B.G.; data curation, C.A., G.N.L. and D.P.; formal analysis, C.A., G.N.L., S.G., S.S., D.P., M.S.L. and C.G.C.; funding acquisition, C.A., C.A.B., C.A.S. and B.G.; investigation, C.A., P.S., T.F., V.B. and M.F.B.P.; methodology, C.A., G.N.L., P.S., T.F., V.B., M.F.B.P., H.H.M., S.S., D.P., M.S.L. and C.G.C.; project administration, C.A., C.A.S. and B.G.; resources, C.A. and C.A.S.; software, C.G.C. and C.A.B.; supervision, C.A., H.H.M. and B.G.; visualization, C.A., G.N.L., S.G., M.S.L. and B.G.; writing—original draft, C.A., S.G. and B.G.; writing—review and editing, C.A., S.G., P.S., T.F., V.B., M.F.B.P., H.H.M., S.S., D.P., M.S.L., C.G.C., C.A.B. and C.A.S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was approved local ethics committee (protocol #37460620.8.0000.0068) and registered at ClinicalTrials.gov (NCT04659486).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Access to de-identified data or related documents can be requested through submission of a proposal with a valuable research question, necessary data protection plan, and ethical approvals. Data requests should be addressed to the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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4.4 Article 4: Exercise in Pediatric COVID-19: A Randomized Controlled Trial

ORIGINAL INVESTIGATION

Exercise in Pediatric COVID-19: A Randomized Controlled Trial

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Abstract

Purpose: This study assessed the impact of a 12-week, home-based exercise training (HBET) program on health-related quality of life (HRQOL, primary outcome), cardiovascular and metabolic parameters in pediatric COVID-19 patients.

Methods: In this single-center, randomized controlled trial conducted in Sao Paulo (Brazil) from October 2020 to January 2022, 32 patients (mean age 12 ± 3.3 years; median 4 months [range: 0.7–6.6] between COVID-19 diagnosis [n = 25 mild, n = 4 moderate, n = 3 severe illness] and study entry) from a tertiary hospital were randomly assigned to either HBET or standard of care (CONTROL) in a 2:1 ratio. The HBET group underwent supervised and unsupervised sessions 3 times/week for 12 weeks emphasizing aerobic and bodyweight exercises and the CONTROL group received standard care which included general advice for a healthy lifestyle with no prescribed exercise intervention. HRQOL (Pediatric Quality of Life Inventory [PedsQL], cardiopulmonary exercise test (CPET), brachial flow-mediated dilation (b-FMD) and echocardiography assessments were conducted in both groups. Statistical analysis was performed using an intention-to-treat approach (ITT) for the primary analysis and complete-case (per-protocol) as sensitivity analysis and significance was set at $P \leq 0.05$ ($P \leq 0.10$ were considered as trend).

Results: There was no difference in HRQOL between groups. Intention-to-treat analysis showed a trend toward increased VO_2 at anaerobic threshold at post-intervention for the HBET group. Additionally, a sensitivity analysis showed significant changes in peak HR, HRR_{1min}, RER and chronotropic response and tendency towards significance in VE/MVV and chronotropic response for the HBET group. No other between-group differences were detected for CPET, b-FMD and echocardiography variables (all $p > 0.05$).

Conclusions: In this RCT, a 12-week HBET intervention did not impact HRQOL in pediatric COVID-19 patients. However, HBET led to greater improvements in VO_2 VAT, heart rate peak and one-minute recovery and in chronotropic response with no changes in other cardiovascular parameters. Further studies are needed to explore the effects of exercise on the recovery of pediatric COVID-19 patients with persistent COVID-19 symptoms.

Exercise in pediatric COVID-19: a randomized controlled trial

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ABSTRACT

PURPOSE: This study assessed the impact of a 12-week, home-based exercise training (HBET) program on health-related quality of life (HRQOL, primary outcome), cardiovascular and metabolic parameters in pediatric COVID-19 patients. **METHODS:** In this single-center, randomized controlled trial conducted in Sao Paulo (Brazil) from October 2020 to January 2022, 32 patients (mean age 12±3.3 years; median 4 months [range: 0.7–6.6] between COVID-19 diagnosis [n=25 mild, n=4 moderate, n=3 severe illness] and study entry) from a tertiary hospital were randomly assigned to either HBET or standard of care (CONTROL) in a 2:1 ratio. The HBET group underwent supervised and unsupervised sessions 3 times/week for 12 weeks emphasizing aerobic and bodyweight exercises and the CONTROL group received standard care which included general advice for a healthy lifestyle with no prescribed exercise intervention. HRQOL (Pediatric Quality of Life Inventory [PedsQL], cardiopulmonary exercise test (CPET), brachial flow-mediated dilation (b-FMD) and echocardiography assessments were conducted in both groups. Statistical analysis was performed using an intention-to-treat approach (ITT) for the primary analysis and complete-case (per-protocol) as sensitivity analysis and significance was set at $P \leq 0.05$ ($P \leq 0.10$ were considered as trend). **RESULTS:** There was no difference in HRQOL between groups. Intention-to-treat analysis showed a trend toward increased VO_2 at anaerobic threshold at post-intervention for the HBET group. Additionally, a sensitivity analysis showed significant changes in peak HR, $HRR_{1min,}$ RER and tendency towards significance in VE/MVV and chronotropic response for the HBET group. No other between-group differences were detected for CPET, b-FMD and echocardiography variables (all $p>0.05$). **CONCLUSION:** In this RCT, a 12-week HBET intervention did not impact

HRQOL in pediatric COVID-19 patients. However, HBET led to greater improvements in VO_2 VAT, heart rate peak and one-minute recovery and in chronotropic response with no changes in other cardiovascular parameters. Further studies are needed to explore the effects of exercise on the recovery of pediatric COVID-19 patients with persistent COVID-19 symptoms.

ClinicalTrials.gov: NCT04659486

Keywords (5): Health-related quality of life; children and adolescents; SARS-CoV-2; Cardiopulmonary exercise test.

INTRODUCTION

COVID-19 is a complex, multisystemic disease that has affected millions of people worldwide (1). Approximately 15.6 million, representing 17.9% of the overall cumulative confirmed COVID-19 cases were reported among children and adolescents (2). In general, children and adolescents infected by SARS-CoV-2 exhibit either mild symptoms or are asymptomatic and recover within 1-2 weeks of the onset of the disease (3). However, the presence of certain risk factors such as obesity, asthma, diabetes mellitus, congenital heart disease, sickle cell disease, malignancies and immunocompromised conditions increase the risk for a more severe illness (4,5). Although only a small percentage of pediatric patients experienced severe clinical symptoms, the disease has led to a notable number of hospitalizations (2).

In the post-acute phase, COVID-19 may lead to significant health impairments such as reduced functional capacity and muscle mass, psychological morbidities (e.g., anxiety and depression) (6), and decreased sleep quality (7). Additionally, we recently showed that pediatric COVID-19 patients can experience impaired cardiorespiratory fitness and endothelial dysfunction 3-6 months after diagnosis (8). Such abnormalities can negatively affect patient's health-related quality of life (HRQOL).

Regular exercise has been shown to improve numerous clinical parameters in children and adolescents, including in those with health impairments (9–11). For instance, literature is unequivocal on the benefits of exercise on cardiovascular health, respiratory capacity, muscle strength, mental health and immune function, exerting anti-inflammatory

and metabolic effects (12,13). Altogether, these benefits underscore the potential role of exercise in mitigating possible harmful events related to SARS-CoV-2 infection. In support to this, Longobardi et al. showed that a home-based exercise training (HBET) intervention improved physical domains of HRQOL, 30-second sit-to-stand performance, and some persistent symptoms (muscle weakness and myalgia) in adult patients previously admitted to intensive care unit due to COVID-19 (14). However, there is a paucity of data on the effects of exercise interventions in pediatric patients after COVID-19 illness.

The present study aims to assess the effects of a 12-week HBET intervention on HRQOL (primary outcome) and several physiological outcomes (aerobic capacity, brachial endothelial function, cardiac function, metabolic, and inflammatory markers) that can potentially affect overall health and/or wellbeing in a sample of pediatric COVID-19 patients with preexisting chronic conditions, followed at a tertiary hospital.

MATERIAL AND METHODS

Study design

This is a 12-week, single-center, randomized controlled trial conducted between October 2020 to January 2022. The study was approved by the National and Institutional Ethical Committee of Clinical Hospital, CAAE: 37460620.8.0000.0068 and registered at ClinicalTrials.gov (NCT04659486). Written parental consent and child assent were obtained. The manuscript was reported according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines. The detailed description of the study design is illustrated in **Figure 1**.

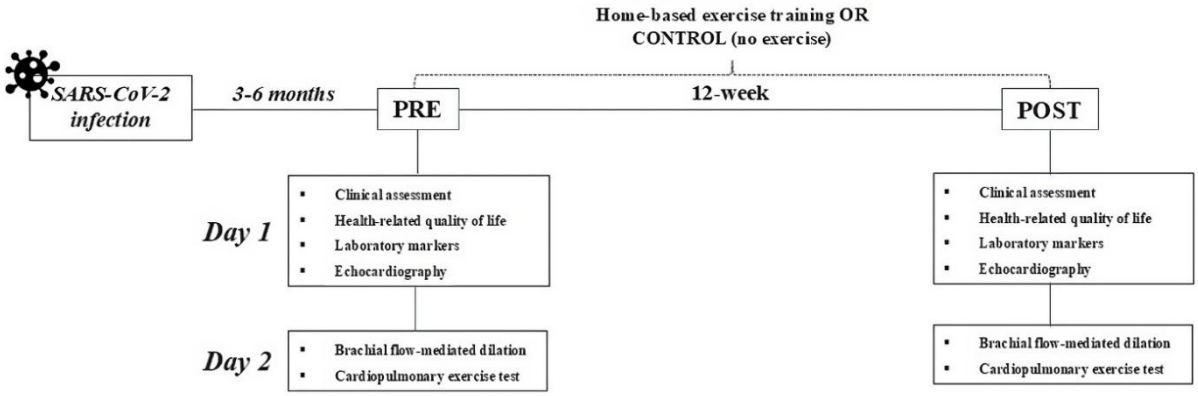

Figure 1. Experimental design.

Randomization

Participants who met the inclusion criteria and accepted to participate were randomly assigned to either HBET or standard of care (CONTROL) groups in a 2:1 ratio after the completion of baseline evaluations, using a computer-generated randomization code in Excel 2021 (Microsoft, Redmond, WA, USA). The process was performed by an independent researcher, who was not involved in conducting the assessments.

Participants

Pediatric inpatients with laboratory-confirmed SARS-CoV-2 from our tertiary hospital (Child and Adolescent Institute from the Clinical Hospital of the School of Medicine of the University of Sao Paulo) that were recruited from October 2020 to August 2021, with a median of 4 months (range: 0.7–6.6 months) between diagnosis and study entry. Preexisting pediatric chronic conditions (>3 months) were diagnosed based on diagnostic criteria and current guidelines (15,16) and classified as 1) autoimmune rheumatic and inflammatory diseases; 2) hepatic and kidney disorders; 3) oncological conditions, and 4) neurological diseases.

Data referring to the acute phase of COVID-19 were retrospectively assessed through medical records. Prospective data were captured through the Research Electronic Data Capture (REDCap) database from the Clinical Hospital of the School of Medicine of the University of Sao Paulo (Brazil) in a dedicated, multidisciplinary, outpatient clinic for COVID-19. Demographic, anthropometric and clinical manifestations (i.e., fever, nasal discharge, sneezing, cough, sore throat, anosmia [loss of smell], dysgeusia [taste alterations], headache, myalgia [muscle pain], arthralgia [joint pain], conjunctivitis, dyspnea, hypoxemia [low oxygen in blood], nausea, vomiting, diarrhea, cutaneous rash, neurological abnormalities, and hospitalization) were assessed during the first study visit. COVID-19 was classified as mild, moderate, severe, or critical (17). Post-COVID-19 symptoms was defined as on-going symptoms 12 weeks or more after acute COVID-19 (18).

The inclusion criteria were as follows: (i) confirmation of SARS-CoV-2 infection by analyzing nasopharyngeal or oropharyngeal swabs using a genomic RNA assay with qRT–PCR or by anti-SARS-CoV-2 IgG serology and/or rapid immunochromatographic assay for anti-SARS-CoV-2 IgM and IgG antibodies and (ii) age at diagnosis between 7 and 18 years

old. Exclusion criteria were children and adolescents who present with diagnoses of multisystem inflammatory syndrome (MIS-C), myocardial dysfunction, refractory cardiac arrhythmias, coronary artery aneurysms with or without thrombi, electrocardiographic alterations suggestive of myocardial infarction or ischemia and clinical signs of heart failure; presence of any limitation or physical disability that prevents the practice of exercise; and pregnancy.

Intervention

Home-based exercise training program

The 12-week, 3-times-a-week, HBET program has been thoroughly described elsewhere (19–21). In brief, exercise sessions had two components. The first one included aerobic exercise predominantly, such as jumping jacks, skipping, high-knees, leg raises and cross jacks. Flexibility and mobility exercises were also included during the first part of the program. The second component included strengthening-oriented bodyweight exercises (push-ups, air squats, lunges, crunches, and planks). One of the three weekly sessions was conducted with real-time online supervision by a fitness trainer, whist the other 2 were unsupervised. Patients were required to provide feedback to the staff upon the completion of the training bout. Training monitoring was conducted through WhatsApp®, Zoom® or Google Meet® apps. The exercise intensity was assessed through the Children's OMNI scale of perceived subject exertion (PSE) ranging from 1-10 during each session (22). Exercise progression was carried out every 4 weeks using the PSE OMNI scale (Level 1: weeks 1-4, PSE: 4-6; weeks 4-8, PSE: 6-7 and weeks 8-12, PSE: 7-8) by modifying the number of repetitions (10 to 15), sets (3 to 4), and/or duration of the sets (30 to 45 seconds). The detailed description of the intervention can be found in **supplementary table 1**. Patients received instructional videos and photos with the prescribed exercise intervention. In order to provide detailed information about the program and collect patient's health status, an initial video call was conducted with patients and their parents prior to the beginning of the program. Adherence to the intervention was assessed using a training log. Subjects assigned to the CONTROL group received standard of care treatment which included general advice for a healthy lifestyle with no prescribed exercise intervention. Whenever necessary, patients

from both groups received outpatient care, consultation with a specialized physician and additional diagnostic exams.

Outcomes

Health-related quality of life (HRQOL) - Pediatric quality of life inventory (PedsQL)

Before and after the intervention, PedsQL (primary outcome) was administered in-person by a trained research staff. This instrument was previously validated for Portuguese language and includes 23-items and four domains: 1) physical functioning (8 items), 2) emotional functioning (5 items), 3) social functioning (5 items), and 4) school functioning (5 items) (23). A five-point response scale was used (0=never a problem to 4=almost always a problem). Items were reverse-scored and linearly transformed to a 0-100 scale (0=100, 1=75, 2=50, 3=25, and 4=0). Higher scores indicated better HRQOL, and scale scores were computed as the sum of the items divided by the number of items answered. If >50% of the items in the scale were missing, the scale score was not computed (24). The physical health summary score (eight items) was the same as the physical functioning subscale. The psychosocial health summary score (15 items) was computed as the sum of the items divided by the number of items answered in the emotional, social, and school functioning subscales.

Cardiopulmonary exercise test (CPET)

Patients performed a maximal graded cardiopulmonary exercise testing on a treadmill (Centurion C200, Micromed, Brazil) using a modified Balke protocol (25), which involves a fixed velocity with a 2% increase in incline every minute, corresponding to an increase of 1 MET per minute to the limit of tolerance; each patient performed the same protocol pre-intervention and post-intervention. Heart rate (HR) was continuously recorded beat-by-beat from the R–R interval using a 12-lead electrocardiograph (ErgoPC Elite, Micromed, Brazil). Gas exchange and ventilatory parameters were recorded breath by breath by continuous sampling using a rapid response gas analyser Metalyzer IIIb/breath-by-breath, Cortex Leipzig Germany (26). System was calibrated immediately before each test following manufacturer’s specifications. Outcome with gas exchange measurement (test termination

was determined by volitional exhaustion, and maximal effort was confirmed by a peak respiratory exchange ratio ≥ 1.0, maximal heart rate > 95% age/gender-predicted values, or maximum effort by the Borg 20-point rate of perceived exertion (RPE) scale (27–29).

The ventilatory anaerobic threshold (VO_{2VAT}) was determined to occur at the breakpoint between the increase in the carbon dioxide output and VO2 (V-Slope) or the point at which the ventilatory equivalent for oxygen reached the minimum value and began to rise without a concomitant rise in ventilatory equivalent for carbon dioxide (30,31). The VO_{2peak} was defined as the maximal attained VO_2 at the end of the exercise period in which the subject refers exhaustion (analog scale of the perceived exertion to Borg scale). To assess ventilatory limitation and efficiency, we measured pulmonary ventilation-to-maximum voluntary ventilation ratio (VE/MVV) and the highest value for end-tidal carbon dioxide pressure ($PETCO_{2max}$, mmHg). Other variables such as oxygen uptake efficiency slope (OUES - L/min; calculated by a linear least squares regression of the VO_2 on the common logarithm of the VE, by using the following equation: VO_2=a log(VE)+b), the VE/VCO_{2nadir} (the lowest VE/VCO_2 ratio), VE/VCO_2 slope (the number of liters of air that must be breathed in order to eliminate 1 liter of carbon dioxide (32,33), O_2 pulse (ml/beat), resting heart rate (HR), peak HR and recovery (i.e., 1 min after exercise; HRR-$_{1min,}$ bpm) and respiratory exchange ratio (RER) were assessed. Chronotropic response during exercise was calculated through the following formula: ([HRpeak-HRrest/220-age-HRrest]×100). The inability to achieve at least 80 percent-predicted heart rate reserve were considered an indicative of impaired chronotropic response (34).

Brachial flow-mediated dilation (b-FMD)

b-FMD was evaluated according to current guidelines (35) using a high-resolution ultrasound (LOGIQ e PRO – GE Healthcare, Chicago, IL, US) equipped with a 4.0–12.0 MHz linear transducer. Initially, participants were positioned in the supine position with their right arm extended at an angle of ~80° from the torso. Longitudinal images of the brachial artery diameter were taken using the B-mode ultrasound, and simultaneous pulse-waved Doppler blood flow velocity was obtained using a 60° insonation angle with the sample volume placed in mid-artery and aligned with the blood flow. Initially, a 1-min

baseline recording of the brachial artery diameter and blood flow velocity was performed. Then, the ischemic stimulus was performed by inflating a cuff placed in the forearm to 60 mmHg above the patient's resting systolic pressure for 5 minutes. Recordings were resumed 60 seconds before cuff deflation and continued for 3 minutes thereafter. Brachial artery diameter and shear rate (4 x mean blood velocity/internal diameter) were analyzed by a blinded evaluator using a semi-automatic edge-detection and wall-tracking software (Cardiovascular Suite, Quipu®, Pisa, Italy). Brachial flow-mediated dilation (%) was calculated as the percentage change of the brachial artery diameter after cuff release in relation to baseline brachial artery diameter [b-FMD = (baseline diameter – peak diameter / baseline diameter) x 100]. The blood flow was calculated as the product of the average flow velocity and the vessel radius (Flow = Vm × πr^2 × 60). The antegrade and retrograde components of blood flow were also computed, representing the area above and below the baseline of the horizontal Doppler axis, respectively. Values lower than the age- and sex-specific 25th percentile (36) was considered as suggestive of endothelial dysfunction.

Echocardiography

A single experienced cardiologist, blinded to the patient assignment group, performed all echocardiographic analyses according to the recommendations of the American Society of Echocardiography and included M-mode, two-dimensional imaging, conventional, and tissue Doppler evaluation at the septal and lateral mitral annulus (37). The equipment used was a Philips Affiniti 70 (Andover, MA 01810 USA), with multifrequency transducers (S 5–1 and S 8–3 MHz). Cardiac chamber dimensions were obtained using two-dimensional mode, and left-ventricle ejection fraction (LVEF) was calculated by Simpson's method (normal LVEF ≥55%) (37). The z-score values for the measures were calculated according to the "Boston Children's Hospital Heart Center (38) and Pediatric Heart Network echo z-score Project" data (39). LV mass (g) was estimated using Devereaux's formula according to the Penn convention and indexed for height (m) raised to an exponential power of 2.7 (37). LV hypertrophy was diagnosed whenever LV mass index was greater than the 95th percentile for sex and age, according to Khoury et al (40). Evaluation of LV diastolic function included mitral E/e' ratio, with e' being the average of values obtained by tissue Doppler at the septal and lateral annulus (normal E/e' <14) (37).

Baseline and follow-up left ventricle systolic and diastolic parameters were compared, using z-scores. The right ventricle systolic function was assessed through Tricuspid annular plane systolic excursion (TAPSE) and dysfunction was detected with the z-score from TAPSE <-2 (41). The extent of global left ventricle myocardial strain in longitudinal axis throughout the cardiac cycle was computed as percentages (GLS%). We considered abnormal a global longitudinal strain less than or equal to –16.7% or at least two segments with a peak systolic strain less than or equal to –16.7% (42,43).

Anthropometry

Body weight and height were assessed using a calibrated digital scale and stadiometer. The same trained technician, blinded to the patients' assignment, conducted all measurements.

Laboratory markers and SARS-CoV-2 testing

We collected blood samples from the median or cephalic basilic vein after a 12-hour fast and analyzed for C reactive protein, D-dimer, ferritin, urea, creatinine, troponin-T, lipid profile and glucose metabolism. SARS-CoV-2 infection was assessed by real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) or antibody testing. Real-time RT-PCR to evaluate SARS-CoV-2 genes was carried-out on swab-collected nasopharyngeal and/or oropharyngeal samples in the Molecular Biology Laboratory of our tertiary hospital (44). Antibodies against the S proteins from the coronavirus spike were performed at our laboratory by two different assays: an enzyme-linked immunosorbent assay for anti-SARS-CoV-2 IgG antibodies and a rapid immunochromatographic assay for anti-SARS-CoV-2 IgM and IgG antibodies (45).

Deviation from the protocol

Inflammatory cytokines and physical activity levels assessed by accelerometers were originally planned, but we had insufficient data collected to proceed with the analysis.

Statistics

Due to the unprecedented nature of the COVID-19 pandemic, no data were available to enable an *a priori* sample size calculation. Therefore, our sample size was determined based on feasibility criteria, considering the availability of eligible patients, resources and research staff following available recommendations (46).

Data are presented as mean, standard deviation (SD), median and interquartile range (IQR) (25-75) and lower and upper 95% confidence interval (95%CI) unless otherwise indicated. Data were analyzed using a linear mixed model with repeated measures, employing a restricted maximum likelihood algorithm. This approach allowed for a robust comparison of longitudinal data, considering potential dropout and missing data through an intention-to-treat (ITT) analysis. The model included 'Group' (comprising HBET and CONTROL) and 'time' (pre-intervention and post-intervention) as fixed factors, while 'patients' were considered a random factor. A post hoc test with *Tukey*'s adjustment was used in case of a significant F value. In addition, we conducted a per-protocol sensitivity analysis, which included only completers. For this analysis, delta changes between groups were compared by independent t-tests. All data analysis was performed using SAS V.9.2 software for Windows and significance was set at $p \leq 0.05$ ($p \leq 0.10$ were considered as trend).

RESULTS

Out of the 88 pediatric COVID-19 patients, 32 were selected and randomized into experimental groups. Two patients from the HBET and one in CONTROL group were lost during follow-up (**figure 2**). Adherence to the intervention was 50% (58% for supervised and 46% for online sessions). No adverse events were reported throughout the intervention period.

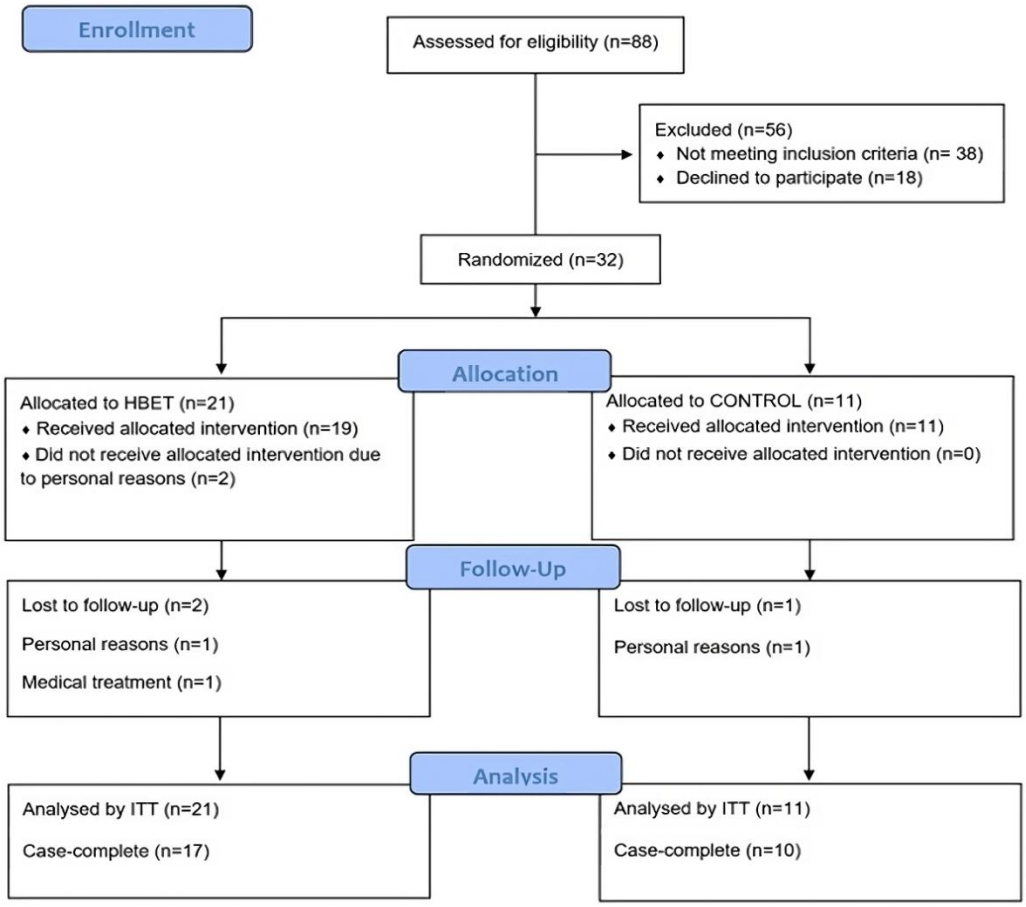

Figure 2. Consort flow diagram. CONSORT, Consolidated Standards of Reporting Trials; CONTROL, standard of care; HBET, home-based exercise training.

Table 1 shows participants' main characteristics at the time of the COVID-19 diagnosis. The mean age of participants was 12±3.3 years, and a significant proportion (n=26, 81%) had preexisting pediatric chronic conditions. In our sample, the majority of COVID-19 cases were categorized as mild (n=25, 78%), with a smaller portion classified as moderate (n=4, 12.5%) and severe (n=3, 9.5%). Also, twenty out of thirty-two had a positive real-time RT-PCR test, and 12 (37%) had a positive serologic test (ELISA assay to detect IgG for SARS-CoV-2).

Table 1. Patients' characteristics at the time of the diagnosis.

Variables	HBET (n=21)	CONTROL (n=11)
Age at hospital discharge, years	12 (8-18)	14 (8-18)
Girls, n (%)	11 (52)	9 (82)
Weight, kg	46±15	59±26
Height, m	1.5±0.2	1.6±0.15
Body mass index ($kg \cdot m^{-2}$)	20±4	24±8
Body surface area, m^2	1.4±0.3	1.5±0.4
Symptoms during acute phase, n (%)		
Fever	12 (57)	6 (54)
Nasal discharge	8 (38)	6 (54)
Sneezing	3 (14)	2 (18)
Cough	8 (38)	6 (54)
Sore throat	4 (19)	4 (36)
Anosmia	4 (19)	1 (9)
Dysgeusia	4 (19)	NR
Headache	8 (31)	6 (54)
Myalgia	5 (24)	3 (27)
Arthralgia	3 (14)	NR
Dyspnea	5 (24)	3 (27)
Hypoxemia	1 (5)	1 (9)
Nausea	4 (19)	2 (18)
Vomiting	2 (10)	1 (9)
Diarrhea	3 (14)	2 (18)
Abdominal pain	2 (10)	1 (9)
Hospitalization	6 (28)	4 (36)
Length of hospital stay (days)	3.5 (1-11)	2 (1-9)
Post-COVID symptoms*	10 (47)	2 (18)
Pre-existing chronic medical diseases, n (%)		
Autoimmune rheumatic and inflammatory diseases	8 (38)	5 (46)
Hepatic and kidney disorders	5 (24)	2 (18)
Oncological conditions	4 (19)	0 (0)
Neurological diseases	0 (0)	2 (18)
None/previously healthy	4 (19)	2 (18)
Pharmacological therapy, n (%)		
Glucocorticoids	1 (5)	3 (27)
Biologics	4 (19)	3 (27)
Immune-system suppressant	2 (10)	0 (0)
Pediatric COVID-19 classification, n (%)		
Mild	16 (76)	9 (82)
Moderate	3 (14)	1 (9)
Severe	2 (10)	1 (9)

Results are presented in n (%), median (minimum-maximum values) or mean (standard deviation) values.
HBET: Home-based exercise training; NR: not reported. *Post-COVID symptoms was defined as on-going sy
12 weeks or more after acute
COVID-19.

After 12 weeks, neither global nor specific domains within the PedsQL scores were significantly different between groups either at baseline or after the intervention (all p>0.05, **table 2**) CPET, b-FMD and echocardiography data are depicted in **table 3**. ITT analysis revealed a trend toward between-group differences at post-intervention for VO_{2VAT} (EMD: -3.66 mL·kg-1·min-1, 95%CI -7.39 to 0.05, p=0.05) in favor of HBET group. Complete-case analysis showed greater changes RER (EMD: 8.66, 95%CI 3.34 to 13.9, p<0.01), HRR_{1min} (EMD: 7.01 bpm, 95%CI -29.1 to -0.13, p=0.04) and peak HR (EMD: 5.68 bpm, 95%CI -24.8 to -3.5, p=0.02), in HBET group compared to CONTROL (**figure 3**, panel L, N and O). Additionally, a trend for greater improvements in VE/MVV (EDM: 34.7, 1.79 to 67.77, p=0.06), chronotropic response (EMD: 16.2%, 95%CI -58 to -10.6, p=0.07) in HBET were also observed (**figure 3**, panel F and P). There were no between-group differences in the other CPET, b-FMD, echocardiography, anthropometry and laboratory parameters in either the ITT or complete-case analyses after 12-week (all p>0.05, table 3 and **supplementary table 2**).

Table 2. Effects of home-based exercise intervention (HBET) and standard of care (CONTROL) on health-related quality of life in pediatric COVID-19 patients, pre- (at baseline) and post-intervention (after 12 weeks).

	HBET (n=21)		**CONTROL (n=11)**		**Post-intervention between-group differences**		
Domains	Baseline	Post	Baseline	Post	EMD	95%CI	P value
PedsQL score (0-100)							
Global	63.6 (55.8; 71.5)	68.4 (61.7; 75.1)	60.7 (46.7;80.5)	68.1 (58;78.3)	1.25	(-11.5;14.0)	0.99
Physical Function	59.3 (51;67.7)	63.3 (56.6; 70)	57.3 (34.2;44.2)	64.5 (52.4;76.5)	1.62	(-6.88;21.1)	0.72
Emotional Function	63.5 (52.9; 74.2)	69.4 (57.8; 81)	61.8 (43.6;80.0)	69.1 (53.8;84.3)	1.59	(-18.8;20)	0.99
Social Function	73.3 (63.6; 82.9)	77.3 (71.4; 83.2)	71 (52.2;89.5)	78.6 (68.6;88.6)	2.71	(-12;17.5)	0.98
School Function	66.4 (56.5; 76.3)	73.6 (64.6; 82.6)	59.1 (41;77.2)	66.8 (50.6;83)	-3.98	(-21.2;13.2)	0.96
Psychosocial Function	67.7 (58.7; 76.7)	73.5 (65.2; 81.7)	64 (47.2;80.5)	71.5 (58.6;84.3)	0.325	(-15;15.6)	1.00

Data are expressed as mean (lower and upper 95%CI). HBET: Home-based exercise training; PedsQL: Pediatric quality of life inventory; CI: confidence interval.

Table 3. Effects of home-based exercise intervention (HBET) and standard of care (CONTROL). Cardiopulmonary exercise test, brachial flow-mediated dilation and echocardiography in pediatric COVID-19 patients, pre- (at baseline) and post-intervention (after 12 weeks).

	HBET (n=21)		**CONTROL (n=11)**		**Post-intervention between-group differences**		
Variables	Baseline	Post	Baseline	Post	EMD	95%CI	P value
Circulation							
Resting heart rate (bpm)	104.1 (96.3;111.8)	94.7 (87;102.4)	99.1 (89.9;108.4)	98.8 (88.8;108.7)	4.01	(-12.7;20.7)	0.91
Peak heart rate (bpm)	178.5 (170.4;186.5)	192.7 (185.8;199.5)	189.4 (188.3;190.3)	183 (170.8;195.1)	-9.7	(-27.3;7.96)	0.44
HRR_{1min} (bpm)	163.3 (152.4;174.2)	163.9 (125.8;174.9)	172.1 (162.7;181.6)	154.9 (142.1;167.6)	-9.04	(-31.9;13.9)	0.70
Performance							
RER	1.09 (1.04;1.14)	1.13 (1.08;1.17)	1.09 (1.04;1.14)	1.04 (0.98;1.10)	0.10	(-0.18;0.01)	0.10
VAT exercise time (min)	4.22 (3.39;5.04)	5.12 (4.50;5.75)	2.78 (1.85;3.7)	4.05 (3.04;5.05)	-1.17	(-2.83;0.48)	0.23
VO_2 VAT ($mL \cdot kg^{-1} \cdot min^{-1}$)	15.9 (14.5;17.4)	18.1 (15.9;20.3)	14.1 (11.7;16.5)	14.2 (11.9;16.5)	-3.66	(-7.39;0.05)	0.05*
Peak exercise time (min)	10.0 (9.03;11.1)	10.4 (8.9;11.9)	9.38 (6.66;12.1)	9.76 (8.03;11.5)	-0.48	(-3.6;2.64)	0.97
VO_2 peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	29.8 (26.6;33)	34.0 (29.4;38.5)	25.5 (20.1;31)	26.9 (20.6;33.3)	-6.53	(-14.9;1.86)	0.16
% Predicted VO_2 peak	60.6 (55;66.4)	69.7 (61.6;77.8)	54.5 (42.8;66.1)	56.6 (45.5;67.8)	-6.62	(-14.3;1.10)	0.11
Ventilatory and gas exchange response							
Peak O_2 pulse (mL/bpm)	8.1 (6.97;9.31)	9 (7.35;10.6)	8.36 (6.82;9.90)	9.3 (7.45;11.1)	0.14	(-2.61;2.90)	0.99
OUES (units/kg)	1.58 (1.37;1.79)	1.62 (1.30;1.94)	1.67 (1.35;1.98)	1.70 (1.26;2.13)	-0.003	(-0.56;0.55)	1.00
VE at VAT (L/min)	22.2 (22.6;25.6)	24.7 (20.5;28.9)	19.8 (15.5;24)	24.7 (20.6;28.8)	0.17	(-7.33;7.68)	0.99
VE at peak (L/min)	59.2 (48;70.4)	73.2 (57;89.4))	58.2 (45.8;70.6)	62.6 (47.2;78)	-10.3	(-36.8;16.1)	0.71
VE/MVV (L/min)	0.54 (0.47;0.61)	0.71 (0.63;0.8)	0.56 (0.41;0.71)	0.58 (0.45;0.70)	0.13	(-0.04;0.32)	0.27
VE/VCO_2 slope (units)	29.1 (27.8;30.3)	29.3 (27.7;30.9)	28.2 (26.3;30.1)	30.8 (28.6;32.9)	0.22	(-4.81;5.25)	0.99
$PETCO_2$ max (mmHg)	35.5 (34.4;36.6)	30.7 (28.9;32.5)	34.7 (32.4;36.9)	30.4 (27.9;32.8)	-0.35	(-3.81;3.1)	0.99
Chronotropic response (%)	89.2 (79.4;99)	104.4 (96.9;112)	99.8 (85;114.6)	92.8 (78.3;107.3)	-11.7	(-33;9.55)	0.44
Doppler ultrasound of the brachial artery							
Basal diameter (mm)	3.20 (2.95;3.45)	3.16 (2.92;3.39)	3.19 (2.99;3.39)	3.02 (2.59;3.46)	-0.07	(-0.71;0.55)	0.98
Peak diameter (mm)	3.38 (3.10;3.65)	3.40 (3.11;3.69)	3.37 (3.18;3.56)	3.18 (2.81;3.54)	-0.17	(-0.87;0.53)	0.90
b-FMD (%)	5.50 (4.83;6.17)	7.67 (5.46;9.88)	5.65 (3.53;777)	5.05 (-0.76;10.8)	-2.38	(-6.61;1.83)	0.41
Shear rate total, $_{AUC}$	126456.47 (89809.10;163163.84)	213803.00 (146882.85;280723.15)	137644.44 (87967.43;187321.46)	187723.25 (75751.36;299695.14)	-23283	(-150725;104159)	0.96

Table 3 – to be continued.

Table 3 – continuation.

Shear rate peak, $_{AUC}$	42360.53 (26547.34;58173.72)	6132.58 (39186.05;83463.12)	52679.11 (33704.21;71654.01)	58691.25 (31519.97;85862.53)	-2196.37	(-49077;44684)	0.99
Mean flow (mL/min)	116.856 (94.048;139.664)	200.778 (98.404;303.152)	139.244 (89.743;188.745)	164.005 (98.474;229.535)	-36.7699	(-192.54;119.00)	0.91
Positive flow (mL/min)	122.823 (100.365;145.281)	214.603 (115.030;314.175)	142.958 (94.285;191.632)	166.965 (99.574;234.355)	-47.4225	(-199.38;104.53)	0.82
Negative flow (mL/min)	-5.9676 (-10.207;-1.728)	-13.824 (-21.751;-5.8972)	-3.716 (-7.872;0.4389)	2.9600 (-7.7647;1.844)	10.7417	(-3.8813;25.364)	0.20
Echocardiography							
LVEDd (z-score)	0.87 (-0.18;1.92)	0.42 (-0.42;1.27)	-0.68 (-1.70;0.34)	-0.77 (-1.75;0.19)	-0.84	(-2.17;1.01)	0.59
LVESd (z-score)	0.41 (-0.90;1.74)	-0.25 (-1.41;0.9)	-0.53 (-1.79;0.72)	-0.95 (-1.83;-0.07)	-0.24	(-2.64;2.14)	0.99
E/A ratio (z-score)	-0.74 (-1.16;-0.31)	-0.72 (-1.06;-0.38)	-1.15 (-2.29;-0.004)	-0.98 (-3.09;1.13)	-0.14	(-1.52;1.23)	0.99
E/e ratio (z-score)	0.10 (-0.53;0.74)	0.35 (-0.20;0.92)	-0.81 (-1.33;-0.28)	-0.20 (-1.34;0.93)	-0.56	(-1.92;0.79)	0.66
TAPSE (cm)	2.18 (1.91;2.44)	2.06 (1.76;2.37)	2.18 (1.94;2.18)	2.25 (1.89;2.60)	0.21	(-0.39;0.81)	0.77
IVSd (mm)	8.29 (7.52;9.06)	8.38 (7.70;9.07)	8.11 (7.30;8.92)	9 (6.81;11.2)	0.64	(-1.23;2.53)	0.78
LVPWd (mm)	3.38 (-2.54;9.3)	8.81 (7.93;9.68)	1.94 (-7.53;11.4)	7.75 (6.88;8.61)	-1.13	(-11.6;9.41)	0.99
LVEF (%)	57.8 (45.1;70.4)	67.8 (59.6;76.1)	59.4 (43.5;75.3)	70 (62.8;77.1)	1.78	(-20.6;24.1)	0.99
GLS (%)	-20.7 (-22;-19.5)	-19.8 (-21.4;-18.5)	-20.7 (-22;-18.2)	-21 (-24.2;-17.7)	4.81	(-13.0;22.6)	0.87

Data are expressed as mean (lower; upper 95% confidence interval). HBET: Home-based exercise training; VAT: Ventilatory anaerobic threshold; VO_2: oxygen uptake; OUES: oxygen uptake efficiency slope; VE: minute ventilation; MVV: maximum voluntary ventilation; VE/VO_2: ventilatory equivalent for carbon dioxide production; VE/VCO2: carbon dioxide production; $PETCO_2$: end-tidal carbon dioxide tension. b-FMD: brachial flow-mediated dilation; AUC: area under curve; LVEDd: left ventricle end-diastolic dimension; LVESd: left ventricle end-systolic dimension; E/A: early to late diastolic flow velocity ratio; E/e: velocity of transmitral flow and E velocity from tissue; TAPSE: tricuspid annular plane systolic excursion; LVEF: left ventricular ejection fraction; GLS: global longitudinal strain. * Indicates significant post-intervention between-group differences ($p<0.05$).

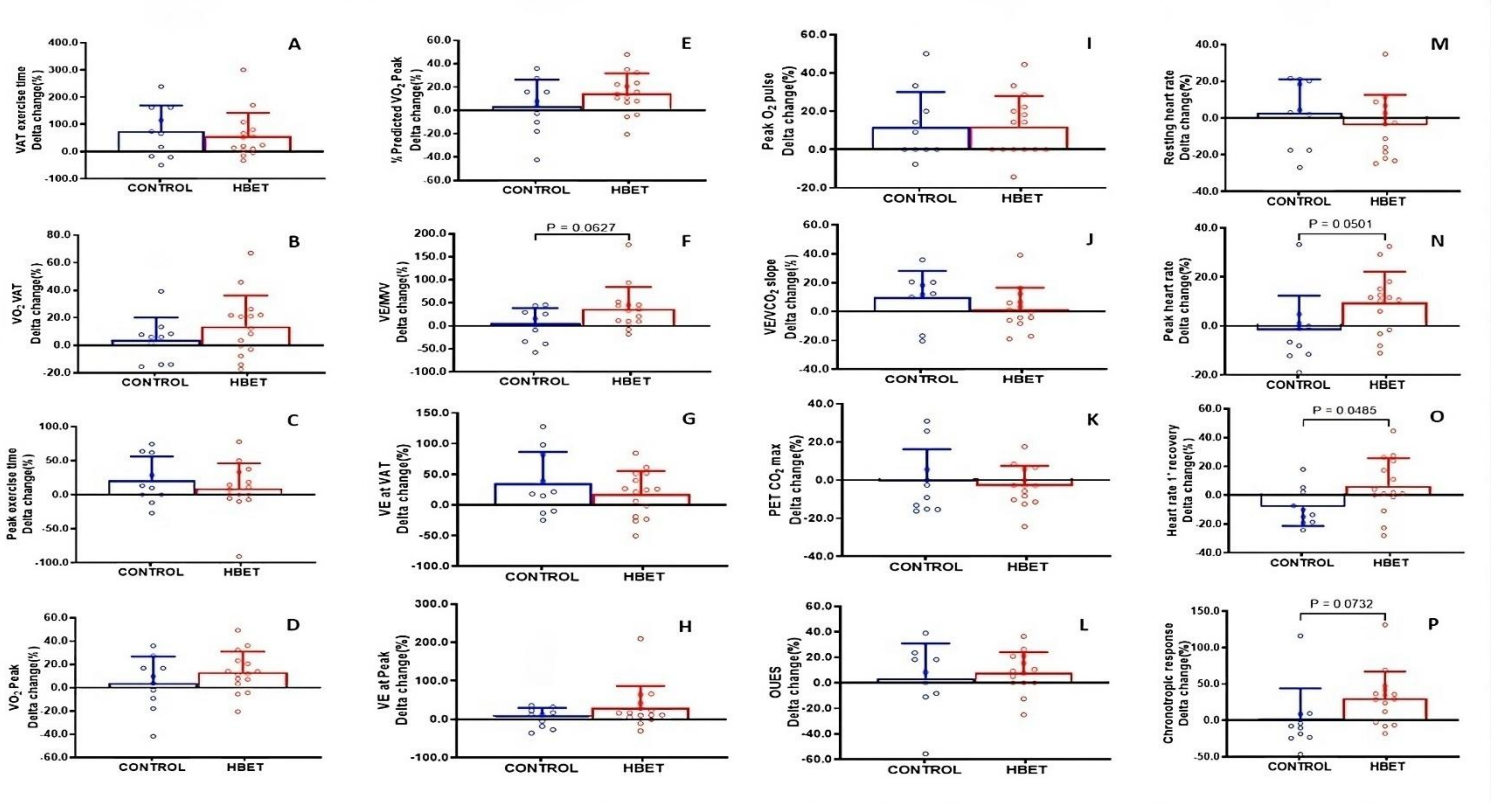

Figure 3 - Cardiopulmonary exercise test parameters after 12 weeks of HBET intervention or standard of care (CONTROL). The reported p-values are derived from a complete-case analysis, utilizing the Mann-Whitney test to compare delta changes between groups. CONTROL, standard of care; HBET, home-based exercise training; VAT: Ventilatory anaerobic threshold; VO_2: oxygen uptake; OUES: oxygen uptake efficiency slope; VE: minute ventilation; MVV: maximum voluntary ventilation; VE/VO_2: ventilatory equivalent for carbon dioxide production; VE/VCO_2: carbon dioxide production; $PETCO_2$: end-tidal carbon dioxide tension**.**

DISCUSSION

To the best of our knowledge, this is the first randomized controlled trial investigating the effects of an exercise intervention in pediatric COVID-19 patients, particularly in those with preexisting chronic conditions. The main positive effect was the improvement of cardiopulmonary parameters such as $VO_{2VAT,}$ peak heart rate, HRR1min and chronotropic reserve in the HBET group. Also, no adverse events were reported. However, changes in HRQOL, the primary outcome, were not observed.

Self-perceived HRQOL is a multidimensional construct that is influenced by various physical and mental factors [10, 17]. COVID-19 infection can significant impact HRQOL. For example, Chen *et al* observed that the hospital length of stay was associated with decreased HRQOL (47). To mitigate this decline, exercise interventions have demonstrated its efficacy in enhancing quality of life across diverse populations (48–50), including individuals infected with COVID-19 (14). However, adherence to interventions plays a crucial role to show the effectiveness of exercise. In agreement, Tarakci *et al* investigated the effects of a HBET in patients with juvenile idiopathic arthritis, an autoimmune inflammatory disorder that affected 13/32 (40%) of patients included in our cohort (15,16). The authors specifically analyzed data from patients who completed at least 75% of the proposed sessions, and they found significant improvements in quality of life and functional ability following the intervention. Also, we have recently reported greater improvements in HRQOL among adult survivors of severe-to-critical COVID-19 who underwent HBET compared to those receiving standard of care alone. Our sensitivity analysis focusing on completers revealed greater enhancements in functional capacity and cardiopulmonary variables favoring HBET (14). Therefore, the low magnitude of change observed in our pediatric sample may be attributed to the low adherence (~50%) and lower severity of the disease during acute phase that could provide a smaller window for improvements. Additionally, COVID-19 had no substantial impact in HRQOL as observed scores at baseline were close to commonly seen for healthy individuals (51), which may have imposed a ceiling effect for this outcome.

Despite the absence of measurable effects in the primary outcome, HBET positively affected some CPET variables. COVID-19 infection may directly impact the cardiovascular

and respiratory systems (52). In a comprehensive review, Schwendinger *et al* documented multisystemic impairments in adult patients recovering from COVID-19 (53). The authors found evidence for ventilatory inefficiency, lower cardiac output, abnormal chronotropic responses, which can compromise the oxygen transport (i.e., convective and diffusive oxygen transport) and utilization capacity, thereby negatively impacting both cardiorespiratory function and exercise tolerance. Importantly, exercise training has proven effective in mitigating the cardiopulmonary side effects associated to SARS-CoV-2 infection and enhance overall health (14,54). For example, our analysis showed that exercised patients increased their peak heart rate and improved chronotropic response after the intervention. It is possible that exercise training led to a better convective oxygen transport and autonomic modulation, thus improving the chronotropic response and exercise tolerance in these patients. In addition, we have also observed a greater HRR1min response, which could indicate an enhanced vagal reactivation by the autonomic nervous system. These findings hold significant importance, as the inability to decrease heart rate after exertion and a blunted heart rate response during exercise have both been linked to increased risk of all-cause mortality across a variety of asymptomatic and diseased populations (55,56). Additionally, increases in RER, combined with improvements in peak heart rate and chronotropic response, suggest that the HBET group was able to tolerate higher intensities of exercise. This is likely because the exercise intervention led to improvements in aerobic capacity, specifically VO_{2VAT}, allowing participants to perform at higher intensities before reaching fatigue.

Previous studies with middle-aged and older adults observed worse metabolic responses to exercise following mild-to-severe COVID-19 (57,58). VO_{2VAT} reflects the onset of metabolic acidosis during exercise that results into elevated arterial lactate levels and heightened ventilatory drive, thus it is considered an indicator the aerobic fitness (59). As it marks the point when anaerobic energy production increases, VO_{2VAT} delineates the upper boundary of a spectrum of exercise intensities that can be predominantly achieved aerobically (60). Notably, the observed mean values for VO_{2VAT} within our sample were far below the reference values for pediatric population (61). Our findings indicate a trend for enhanced VO_{2VAT} by exercise training, suggesting that HBET can effectively improve oxidative metabolism in pediatric COVID-19 patients. Children have a reduced trainability

compared to adults, suggesting the presence of a maturational threshold in young individuals' responses to exercise (62). However, some exercise-induced adaptations have been reported in youth, such as improvements in exercise economy, $VO_{2VAT,}$ oxygen uptake and a better ventilatory efficiency, particularly following in-person, supervised training programs (63–65). Improvement in VO_{2VAT} after our home-based protocol align with these observations, but the relatively low magnitude of change suggests that the stimulus promoted by HBET may have been suboptimal to produce more pronounced adaptations in this and other CPET outcomes.

Both physical activity and physical inactivity can trigger significant and contrasting alterations in vascular function (66–68). For instance, do Amaral *et al* found that older adults who were previously hospitalized with COVID-19 experienced significant improvements in carotid-femoral pulse wave velocity, a prognostic marker of cardiovascular function, after undergoing HBET (69). In contrast, we failed to detect any effect of our intervention on b-FMD and echocardiography parameters, contrary to our initial expectations. The absence of data regarding patients' physical activity levels limits the interpretation of our findings. Considering the pandemic context, where many patients remained confined to their homes, it is likely that sedentary behavior prevailed during other periods of the day, which may have counteracted any potential benefits of exercise (70). Also, part of the discrepancies is likely related to age differences between the studied populations (i.e., older adults vs. children). Further studies involving pediatric populations are necessary to confirm the present findings.

The present study has several limitations. First, the sample size was low and probably underpowered to detect significant changes for some outcomes; the high heterogeneity of the sample may also have contributed to type II error. Second, it is possible that the low adherence to the protocol might have contributed to some null findings. Third, we could not determine the specific SARS-CoV-2 variant responsible for patients’ infections; however, our sample was recruited and randomized during the same period in which the Gamma variant predominated in Brazil (71). Additionally, the potential negative impact of the COVID-19 pandemic on the physical activity levels of control participants may have influenced our outcomes. This data cannot be extrapolated to other pediatric populations or to patients with more severe forms of COVID-19 (e.g., those suffering from MIS-C).

Likewise, generalizations for other types of exercise interventions and delivery (e.g., in person) must be avoided.

CONCLUSION

HBET intervention did not impact HRQOL in a small sample of pediatric COVID-19 patients, but despite the low participants' adherence, exercise was able to improve heart rate recovery and chronotropic reserve (also considering complete-case analysis), which are reflective of a better autonomic function. Given the paucity of data involving pediatric patients who had COVID-19, these preliminary findings can help to design further exercise interventions focused on recovering functionality and wellbeing in this population.

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SUPPLEMENTARY FILES

Supplementary table 1 - Overview of the Home-Based Exercise Program: exercise components, performance guidelines, adaptations, volume, intensity, and Perceived Subjective Effort (PSE) for pediatric COVID-19 patients.

Level	Exercise Component	Exercises	Performance Points	Adaptations* (for patients with certain level of physical limitation)	Volume	Intensity	OMNI scale for PSE[1]
1	**Warm-Up**	1. Skipping 2. Knee raises 3. Jumping jacks 4. Heel lifts	1. Coordinate arm with opposite leg. 2. Lift knee to hip line. 3. Clap hands above head. 4. Try to touch heel to buttocks.	1. Use wall for support. 2. Perform slower until moderate intensity is reached. 3. Simplify by opening and closing feet without moving. 4. Do not let heel touch the buttocks.	3x30 sec	Low	1-2 week: 4-5 3-4 week: 5-6
	Main Part	1. Half squats 2. Short crunches 3. Push-ups with knees 4. Static lunges 5. Front plank	1. Feet shoulder-width, maintain lumbar curve. 2. Coordinate breathing, avoid lumbar curve. 3. Keep elbows in line with shoulders. 4. Keep feet and knees forward. 5. Keep elbows in line with shoulders.	1. Use chair for support. 2. Anchor feet under bed/sofa. 3. Use wall or sofa for support. 4. Start with wall support. 5. Plank with extended arms or on knees.	3x12 reps		

2	**Warm-Up**	1. Toe touches 2. Frankenstein walk 3. Jumps in place 4. Mountain climbers	1. Try to touch opposite foot with hand. 2. Mimic Frankenstein motion. 3. Start in half-squat, increase difficulty. 4. Move knee towards opposite elbow.	1. Touch same side foot. 2. Perform leg raises instead. 3. No adaptations needed. 4. Increase speed gradually.	3x40 sec	Moderate	5-6 week: 6 7-8 week: 6-7
	Main Part	1. Full squats 2. Triceps dips on chair 3. Side plank 4. Step-ups 5. Sit-up rower	1. Keep knees behind toes. 2. Keep elbows at 90°. 3. Keep spine straight, head neutral. 4. Ensure chair is stable. 5. Coordinate breath with movement.	1. Reduce squat depth. 2. Perform dips on floor. 3. Use wall for support. 4. Reduce step height. 5. Increase speed gradually.	4x10 reps		

3	**Warm-Up**	1. Hip stretch 2. Lateral jumps 3. Jumping jacks 4. Long jumps	1. Start in prone, bring knee to chest. 2. Jump over object (e.g., shoe). 3. Clap hands above head. 4. Jump as far as possible.	1. Perform dynamic leg pulls. 2. Perform lateral steps without jumping. 3. Increase speed gradually. 4. Perform distance steps instead.	3x45 sec	High	9-10 week: 7-8 11-12 week: 8
	Main Part	1. Wall sit (maximum duration) 2. Alternating lunges 3. Leg raises crunches 4. Push-up + mountain climber 5. Superman	1. Back against wall, knees at 90°. 2. Ensure forward knee doesn't pass toes. 3. Keep legs straight. 4. Add knee lifts to push-up. 5. Alternate leg and arm lifts.	1. Reduce wall sit depth. 2. Perform slower lunges. 3. Flex knees if needed. 4. Start with knees on floor. 5. Perform dynamic Superman.	4x12 reps		

[1] Robertson RJ, Goss FL, Boer NF, et al. Children's OMNI scale of perceived exertion: Mixed gender and race validation. *Med Sci Sports Exerc*. 2000;32(2) doi:10.1097/00005768-200002000-00029.

Supplementary table 2 - Effects of HBET intervention and standard of care (CONTROL) anthropometry, inflammatory and metabolic parameters in pediatric COVID-19 survivors, pre-intervention (ie, at baseline) and post-intervention (ie, after 12 weeks).

Variables	HBET (n=21)		CONTROL (n=11)		Post-intervention between-group differences		
	Mean Baseline (95CI)	Mean Post (95CI)	Mean Baseline (95CI)	Mean Post (95CI)	EMD	95%CI	P value
Height (cm)	1.51 (1.43;1.58)	1.56 (1.48;1.63)	1.57 (1.46;1.67)	1.56 (1.45;1.68)	0.005	(-0.17 to 0.18)	1.00
Weight (kg)	48 (40.8;54.6)	53.6 (45.4;61.7)	60 (42.3;77.7)	65 (42.9;87)	11.3	(-12.7 to 35.4)	1.00
BMI (kg/m^2)	15.5 (13.7;17.3)	17 (14,7;19)	19 (14;23.7)	20.4 (14.4;23.3)	3.43	(-2.9 to 9.89)	0.83
CRP (0.3–10 mg/L)	2.97 (1.01;4.94)	1.44 (0.67;2.21)	6.97 (-1.86;15.8)	4.28 9 (-2.58;11.1)	2.83	(-5 to 10.6)	0.75
D-dimers (≤500 ng/mL)	416.3 (284.6;548)	457.4 (312.5;602.3)	418.3 9234.6;602.1)	328.9 (224.5;433.2)	-104.2	(-385 to 177)	0.74
Ferritin (10-142 ng/mL)	175.6 (36.2;315)	102.8 (48.5;157.2)	72 (36.3;107.6)	70.7 (36.3;105.1)	-37.1	(-238 to 164)	0.95
Urea (7–20 mg/dL)	22.8 (16.9;28.7)	26.2 (22.1;30.2)	24.8 (20.6;29)	23.3 (18.2;28.3)	-1.05	(-11.2 to 9.10)	0.99
Creatinine (0.59–1.53 mg/dL)	3.28 (-0.90;7.84)	0.62 (0.53;0.72)	0.54 (0.42;0.65)	0.61 (0.50;0.71)	1.42	(-5.27 to 8.11)	1.00
Troponin T (<0.004 ng/mL)	0.004 (0.002;0.007)	0.001 (-0.0002-0.003)	0.004 (-0.003;0.012)	0.002 (-0.001;0.007)	0.001	(-0.005 to 0.008)	0.93
Total cholesterol (<170 mg/dL)	139.3 (128.6;149.9)	135.1 (122.6;147.6)	163.1 (105.5;220.7)	144.2 (129.1;159.3)	8.05	(-35 to 51.1)	0.95
HDL (>60 mg/dL)	52.4 (47.9;56.8)	52.6 (47.9;57.3)	47.8 (32.8;62.7)	45.6 (37.4;53.8)	-7.01	(-20.4 to 6.29)	0.48
LDL (<130 mg/dL)	81.2 (72.3;90)	76.5 (66.9;86.4)	106.6 (62;151.2)	88.3 (78.9;97.8)	10.8	(-22.6 to 44.3)	0.81
VLDL (2-30 mg/dL)	14.1 (10.9;17.3)	13.1 (10;16.2)	18.3 (10;26.7)	18.4 (11.8;25.0)	5.47	(-3.08 to 14)	0.99
Triglycerides (<90 mg/dL)	70.3 (54.4;86.2)	66.2 (50.7;81.7)	94.8 (53.3;136.2)	89.1 (54.6;123.7)	23.7	(-19.4 to 67)	0.44
Glycemia (70-100 mg/dL)	80.7 (77.6;83.9)	80.3 (77.6;82.9)	72.9 (68.8;76.9)	81.2 (75.2;87.2)	0.93	(-6.09 to 7.97)	0.98
Glycated hemoglobin (<5.7%)	5.07 (4.83;5.31)	5.23 (4.97;5.50)	5.01 (4.82;5.21)	5.04 (4.83;5.25)	-0.17	(-0.64 to 0.29)	0.74

Data are expressed as mean (lower and upper 95% confidence intervals). HBET: home-based exercise training; CRP: C-reactive protein; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very-low density lipoprotein.

Declarations

Data availability statement

Access to de-identified data or related documents can be requested through submission of a proposal with a valuable research question, necessary data protection plan, and ethical approvals. Data requests should be addressed to the corresponding author.

Acknowledgments

The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation, and statement that results of the present study do not constitute endorsement by ACSM.

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Conflict of interest disclosure

We declare no competing interests.

Ethics approval statement and clinical trial registration

This study was approved local ethics committee (protocol #37460620.8.0000.0068) and registered at ClinicalTrials.gov (NCT04659486).

Authors' contributions

All authors contributed substantially to the conception and design of the study and in the analysis and interpretation of data. All authors revised the work critically and approved the final version.

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5. GENERAL DISCUSSION

As mentioned, this thesis was part of a larger project called “Prospective studies in schoolchildren and adolescents with COVID-19 treated at HCFMUSP” (see in Annex 1). Our main hypothesis was that a HBET program could enhance the HRQOL and other physiological outcomes of children and adolescents after SARS-CoV-2 infection, particularly those with preexisting chronic conditions followed in a tertiary hospital. Collectively, the four articles included in this thesis offer valuable insights into the impacts of COVID-19 and MIS-C, ranging from a deeper understanding of the infection itself to the evaluation of the effects of exercise-based interventions in the pediatric population.

5.1 Impact of SARS-CoV-2 infection on health outcomes in children and adolescents

While earlier studies demonstrated that underlying chronic conditions significantly increase the risk severe COVID disease and mortality in children and adolescents (67,68), the majority of our patients experienced mild disease, and only 6% (n=2) were classified as severe during acute phase, which are consistent with findings from Wang et al., suggesting that effective baseline management and immunosuppressive therapies may mitigate disease severity (69). Similarly, children with cancer, despite their immunocompromised states, displayed outcomes comparable to the general pediatric population (70). Contrary to our initial concern, pediatric patients with well-managed immunocompromised diseases may not face significantly elevated risks for severe outcomes during acute phase. This discrepancy might be attributed to the effective management of underlying conditions, including immunosuppressive therapies, which may have modulated the inflammatory response.

In contrast, all MIS-C patients in our study were previously healthy, which aligns with findings from Feldstein et al. (2020), who reported that 73% of MIS-C cases occurred in children with no preexisting conditions (71). Four out of five patients required PICU admission, with three experiencing vasodilatory shock and two needing respiratory support. These severe acute presentations are consistent with previous reports (26,71–73), highlighting MIS-C as a hyperinflammatory syndrome leading to multisystem dysfunction, even in set of patients with no preexisting medical condition.

We also observed persistent impairments in cardiorespiratory fitness and endothelial function 3-6 months after diagnosis. Reduced CPET parameters as ventilatory anaerobic threshold and peak oxygen uptake were observed across conditions, highlighting significant aerobic inefficiency. These impairments, seen even in asymptomatic cases, align with studies from Schoeffl et al. (2024) and Denina et al. (2023) which identified similar reductions in exercise capacity attributed to deconditioning, mostly, related to quarantine measures during pandemic rather than structural abnormalities related to the infection (74,75).

Another major finding in our cohort was the impaired FMD, indicating the presence of endothelial dysfunction in both COVID-19 and MIS-C. Several studies have characterized COVID-19 as a vascular disease, where the endothelium plays an important role in complications such as thrombosis, microvascular ischemia, and multiorgan failure (76,77). A recent study demonstrated that reduced FMD values and two primary CPET parameters (i.e., VE/VCO_2 slope and $PETCO_2$ at peak,) were correlated with alterations in endothelial barrier properties in systemic and pulmonary circulation, which may partly explain the reduced ventilatory efficiency observed in COVID-19 patients (78). While these findings suggest a potential link between endothelial dysfunction and impaired exercise capacity, our data is insufficient to fully elucidate the pathophysiological mechanisms underlying these vascular alterations observed in our cohort.

The use of the 13N PET-CT brought valuable insights into the pathophysiological aspects of MIS-C. This advanced technique is recognized as the most accurate and non-invasive tool for assessing microvascular health, allowing precise quantification of CFR in both epicardial and microvascular blood flow. Such measurements are crucial, as impaired CFR has been associated with adverse cardiovascular events, including heart failure and cardiac death (79). Four out of five MIS-C patients exhibited signals of vascular damage, as evidenced by impaired FMD measurements and abnormal CFR values. The endothelial dysfunction observed in our MIS-C patients may have contributed to the microvascular damage reflected in the impaired CFR. However, as this study is based on a case series, our findings cannot establish an association or infer direct causality between endothelial dysfunction and the observed microvascular abnormalities.

Our strengths include the inclusion of a carefully matched control group based on age, sex, BMI, and preexisting conditions, enabling a more robust comparison while minimizing the influence of potential confounding factors. Although we adopted the arbitrary threshold of CFR <2

to define abnormal values, which has not been validated in pediatric populations, the inclusion of CPET and FMD as complementary high-standard methodologies strengthens the assessment of cardiac and vascular outcomes. Our limitations include the cross-sectional design, which precludes the ability to establish causation or evaluate the natural progression of cardiovascular and pulmonary alterations in post-COVID-19 patients, the small sample size, particularly in the case series reduces statistical power and limits generalizability. Potential confounders, including pre-pandemic physical activity levels, dietary habits, and underlying health behaviors, were not assessed, which may limit our ability to fully isolate the specific effects of COVID-19 or MIS-C on the observed abnormalities. Additionally, the absence of baseline values for our cohort prior to infection restricts our ability to determine whether the detected vascular and cardiopulmonary abnormalities were due to the preexisting conditions exacerbated by SARS-CoV-2 or entirely new sequelae resulting from the infection. Collectively, these limitations underscore the need for future longitudinal studies with larger, diverse cohorts and robust methodological designs to confirm and expand upon our findings.

5.2 Feasibility and effects of exercise after SARS-CoV-2 infection

Studies three and four collectively offer valuable insights into the feasibility, efficacy, and challenges of implementing HBET as a rehabilitation strategy, particularly for children affected by severe manifestations like MIS-C. Although the case series design, the third study was the first to suggest improvements in HRQOL, CFR, and cardiorespiratory fitness following a supervised, remotely delivered exercise program in MIS-C. Our findings are particularly relevant due to the limited literature on post-MIS-C rehabilitation.

We initially described microvascular impairments by the low CFR thresholds in our case series. Interestingly, patients who completed the exercise protocol had reduced abnormal CFR in the LV segments, whereas the non-exercised patients (PIV and PVI) increased the distribution of abnormal LV segments. Furthermore, exercised patients also improved their HRQOL and showed greater percentage changes in cardiorespiratory fitness, evidenced by enhanced ventilatory thresholds and VO_2 peak, as well as improvements in %LVEF, highlighting the benefits of the HBET program on both functional and cardiovascular parameters. Notably, Patient III, who exhibited lower adherence to the intervention showed improvements only in select domains of

PODCI and diminished changes in other parameters. The lack of consistent participation may have attenuated the benefits typically associated with structured exercise, such as enhanced ventilatory thresholds and VO_2 peak (80–82), and %LVEF (83), highlighting the possible influence of exercise adherence in the effectiveness of the program. FMD results were not available due to the limited data collected.

In addition, non-exercised patients showed minimal to no improvements in HRQOL, cardiorespiratory fitness, and cardiovascular function after the 12-wk study period. The lack of improvements could be partly attributed to the deconditioning effect, a well-documented phenomenon in patients recovering from severe illness, particularly when physical activity is significantly reduced (84). During hospitalization, MIS-C patients experienced a period of high-inflammatory state, leading to diverse health impairments. Even though their laboratory markers were within the normal range after the study period, prolonged inactivity during and after illness exacerbates declines in aerobic capacity, muscular strength, and cardiovascular efficiency (84–86). Deconditioning not only limits functional recovery but may also amplify the persistent inflammatory and microvascular abnormalities, hindering the natural resolution of these sequelae (87). In addition, no adverse events were reported during the supervised or online exercise sessions, showing the applicability and feasibility of our intervention.

Clinical trials related to the effects of exercise intervention in children and adolescents after COVID-19 remain scarce. For instance, one RCT conducted in our laboratory evaluated the effects of HBET in older adults recovering from moderate to severe COVID-19. Longobardi et al. demonstrated that those who participated in HBET experienced greater improvements in HRQOL and functional capacity compared to those receiving standard care alone (82). In addition, these findings are aligned with the REGAIN study, which evaluated an online, supervised group-based intervention and reported improvements in HRQOL, fatigue, and mental health symptoms among adults with post-COVID syndrome (51).

Although no significant differences were observed in HRQOL, our sensitivity analysis focusing on completers showed significant improvements in cardiorespiratory parameters, including VO_2 at anaerobic threshold, heart rate recovery, and chronotropic response. These findings are further supported by studies on exercise interventions in pediatric populations with chronic respiratory conditions, such as asthma, where combined aerobic and resistance training

programs have been shown to significantly improve cardiorespiratory fitness, oxygen uptake, and muscle strength, even in the absence of changes in lung function or quality of life (52).

The improvements observed in our MIS-C patients, particularly in HRQOL, were likely due to the reduced baseline health impairments, providing substantial room for improvement. This aligns with previous literature indicating that patients with more severe initial impairments often exhibit greater benefits from rehabilitation interventions (51,82,88).

Another important factor that may have limited the ability of our intervention to improve HRQOL and other outcomes was the low adherence seeing in our cohort, with patients completing only 50% of the prescribed exercise sessions. As stated by Nagpal et al., low adherence can undermine the observed effects of an intervention (80). However, even when adherence is low, interventions may still produce positive outcomes, as observed in our study, indicating that exercise, when performed even with minimum threshold produces significant improvements. It is also important to note that various factors could influence participant's adherence to the study protocol, such as presence of chronic illness, environmental and family dynamics, socioeconomics, as well as intrinsic motivators to engage in the intervention.

6. CONCLUSIONS

In conclusion, our finding highlights the multisystemic nature of COVID-19 and MIS-C disease in a sample of mainly immunocompromised and previously health children and adolescents, with significant cardiovascular, pulmonary, and endothelial impairments observed. The feasibility and effectiveness of a HBET program were also demonstrated, showing potential benefits in improving HRQOL, cardiorespiratory capacity, and cardiovascular function, particularly in children recovering from MIS-C.

Together, these studies have not only advanced understanding on the physiological and psychosocial impacts of COVID-19 and MIS-C in children but also represented a significant step forward in addressing the unique challenges posed by the pandemic for pediatric healthcare. However, larger, more diverse cohorts and longitudinal designs are necessary to validate these findings, explore mechanistic pathways, and establish optimal protocols for exercise-based rehabilitation in this population.

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ANNEX 1. Prospective studies in schoolchildren and adolescents with COVID-19 treated at HCFMUSP

Estudos prospectivos nas crianças escolares e nos adolescentes com a COVID-19 tratados no HCFMUSP

Resumo

Introdução: Uma pandemia causada por um novo agente denominado "severe acute respiratory syndrome coronavirus-2" (SARS-CoV-2) e sua doença associada, a "coronavirus disease 2019" (COVID-19), tem acometido raramente crianças e adolescentes, com incidência variando de 1,2% a 2%. No Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP), um recente estudo incluiu 66 crianças e adolescentes que apresentaram a COVID-19, confirmada laboratorialmente. Dois terços destes pacientes apresentaram pelo menos uma condição crônica e 91% sobreviveram a esta doença infecciosa. Crianças escolares e adolescentes sobreviventes com a COVID-19 podem ter inflamação persistente, curso crônico da COVID-19, com agressões isoladas ou concomitantes de vários órgãos e sistemas, tornando esta doença como uma potencial condição crônica, impactando aspectos da qualidade de vida relacionada à saúde (QVRS), saúde física e mental. Além disto, a COVID-19 pediátrica pode induzir autoimunidade (com possibilidade de hipotireoidismo primário e diabetes mellitus tipo I), retardo no crescimento linear e desenvolvimento puberal atrasado, imunodeficiência secundária e apresentar polimorfismos genéticos na plasticidade cerebral impactando na reabilitação. Tendo em vista que crianças escolares e adolescentes com a COVID-19 devam apresentar fraqueza muscular, disautonomia, astenia e sedentarismo, é fundamental que se desenvolvam intervenções seguras e eficazes de manutenção de níveis adequados de atividade física e que possam ser implementados em larga escala. Entretanto, até o presente momento, não há estudos longitudinais sistematizados que avaliaram todos estes aspectos em uma população pediátrica que sobreviveu a COVID-19, particularmente com condições crônicas e que foram hospitalizados em um serviço terciário.

Objetivos: 1) Avaliar possíveis agressões evolutivas aos órgãos (coração, timo, pâncreas e tireoide) em crianças escolares e adolescentes que se recuperaram da COVID-19 *versus* indivíduos que não apresentaram infecção pelo SARS-CoV-2; **2)** Analisar impacto no crescimento e

puberdade, na marcha, assim como imunodeficiência secundária e polimorfismos genéticos na plasticidade cerebral em crianças escolares e adolescentes sobreviventes da COVID-19 comparados aos pacientes que não apresentaram esta condição; **3)** Verificar impacto nos aspectos da QVRS, saúde física e mental em crianças escolares e adolescentes que se recuperaram da COVID-19 *versus* indivíduos que não apresentaram infecção pelo SARS-CoV-2; **4)** Estudar capacidade física, nível de atividade física e consumo alimentar, assim como avaliar a segurança e eficácia em um ensaio clinico randomizado de um programa de treinamento físico aeróbio em pacientes pediátricos sobreviventes da COVID-19.

Métodos: Trata-se de um estudo prospectivo e multidisciplinar que incluirá uma amostra de conveniência de 100 crianças escolares e adolescentes que se recuperaram da COVID-19, tratados no HC-FMSP. As crianças escolares e adolescentes com a COVID-19 terão os seguintes critérios de inclusão: **a)** diagnosticados com a COVID-19 no ano de 2020; **b)** confirmação da infecção pelo SARS-CoV-2 (RT-PCR em tempo real ou por teste de anticorpos); **c)** idade ao diagnóstico até 18 anos e **d)** indivíduo previamente saudável ou com alguma condição crônica preexistente. Este estudo prospectivo e multidisciplinar incluirá 9 projetos: **1)** protocolo clinico e aspectos da QVRS ao diagnóstico e evolutivo da COVID-19 pediátrica; **2)** avaliação prospectiva cardíaca (clinica, biomarcadores de injúria miocárdica e ecocardiograma convencional e pela técnica de *speckle-tracking* bidimensional); **3)** avaliações da imunocompetência, incluindo a função tímica; **4)** avaliações prospectivas de autoimunidade (contra tireoide e pâncreas), do crescimento linear e desenvolvimento puberal; **5)** avaliações prospectivas do metabolismo ósseo e composição corporal em adolescentes; **6)** avaliações da marcha, termografia e polimorfismos genéticos na plasticidade cerebral, impactando na reabilitação; **7)** avaliação da saúde mental e intervenção psiquiátrica breve; **8)** avaliação de capacidade física, nível de atividade física e consumo alimentar; **9)** Ensaios clínicos controlados e randomizados de programas de atividade física

Análises estatísticas: O número amostral dos estudos de intervenção foi determinado com base na viabilidade de recrutamento e seleção de voluntários, e equipe de pesquisa e recursos disponíveis, seguindo recomendações prévias. Os resultados das variáveis contínuas serão expressos em mediana (valores mínimo e máximo) ou média±desvio padrão e comparados pelo teste de Mann-Whitney e t de Student, respectivamente. Os resultados das variáveis categóricas serão apresentados como frequência (porcentagem) e comparados pelo teste exato de Fisher ou qui-

quadrado de Pearson, conforme apropriado. Valores p inferiores a 0,05 serão considerados significantes.

Introdução

Recentemente, uma pandemia causada por um novo agente denominado "severe acute respiratory syndrome coronavirus-2" (SARS-CoV-2) e sua doença associada, a "coronavirus disease 2019" (COVID-19), tem acometido a população mundial e preferencialmente os adultos (1-6). Estudos chineses, italianos e norte-americanos revelaram que raramente a COVID-19 tem sido descrita em crianças escolares e adolescentes, com incidência variando de 1,2% a 2% (7-10).

Uma revisão sistemática evidenciou que ao contrário dos adultos com a COVID-19, a grande maioria das crianças escolares e dos adolescentes infectados com o SARS-CoV-2 tinha uma doença mais branda e os óbitos foram infrequentemente reportados (7). No Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP), um recente estudo incluiu 66 crianças escolares e adolescentes que apresentaram a COVID-19, confirmada laboratorialmente por ensaios em reação em cadeia da polimerase com transcrição reversa em tempo real (real-time RT-PCR) ou por sorologia. Dois terços destes pacientes apresentaram pelo menos uma condição crônica e 91% destes sobreviveram a esta doença infecto contagiosa (6).

O espectro clínico da COVID-19 pediátrica é muito amplo, variando desde pacientes assintomáticos aos gravemente enfermos. Os sinais e sintomas mais comuns em crianças escolares e adolescentes são: febre, tosse, cefaleia, dor de garganta, mialgia, dispneia, conjuntivite, náusea, dor abdominal, vômito e diarreia (2,5-10). Outras características clínicas foram também relatadas em populações pediátricas com a COVID-19, tais como: manifestações cardiovasculares, renais, trombóticas, cutâneas, olfativas, gustativas, neurológicas e oculares. Leucopenia, linfopenia e aumento de marcadores inflamatórios foram as anormalidades laboratoriais mais frequentemente relatadas nesses pacientes (2-10).

Casos graves de uma nova síndrome inflamatória multissistêmica pediátrica em crianças (SIM-P), temporalmente associada à COVID-19 (6, 11-19), foram recentemente descritos, ocorrendo em 9% das crianças e dos adolescentes no HC-FMUSP (6). As características clínicas da SIM-P são muito semelhantes ~~com~~ à síndrome de Kawasaki, síndrome de choque associada à síndrome de Kawasaki, síndrome de ativação macrofágica e síndrome de choque tóxico (6, 11-19).

A SIM-P acomete pelo menos dois órgãos e sistemas, tais como: cardíaco, renal, respiratório, hematológico, gastrointestinal, dermatológico ou neurológico. Vários sinais e sintomas têm sido demonstrados nos pacientes com SIM-P, destacando-se febre persistente, além dos acometimentos cardiovasculares (disfunção miocárdica, miocardite, acometimento de artérias coronárias com sinais ecocardiográficos de vasculite, dilatação e/ou aneurismas), renais (doença renal aguda dialítica), respiratórias (dispneia, taquipneia, hipoxemia), hematológico (trombose localizada ou sistêmica, anemia, leucopenia, linfopenia, plaquetopenia, coagulopatia de consumo), gastrointestinais (dor abdominal, vômito e diarreia), mucocutâneo (edema e fissura de lábios, língua em framboesa, eritema de orofaringe, conjuntivite, exantema polimórfico, vesículas e eritema pérnio) e neurológicas (cefaleia persistente, convulsão, psicose) (6,11-19).

As principais alterações laboratoriais evidenciadas nas crianças e adolescentes com a SIM-P são elevações das provas de atividade inflamatórias (proteína C-reativa, velocidade de hemossedimentação, procalcitonina, ferritina); dos marcadores de coagulopatia (tempo de protrombina, tempo de tromboplastina parcial ativado, D-dímero elevados) e das provas de função miocárdica [troponina, N-terminal do peptídeo natriurético tipo B (NT-proBNP)]. Comprometimento cardiovascular ocorre em até 80% dos casos com SIM-P, principalmente de artérias coronárias (dilatação e aneurismas), disfunção miocárdica, infarto agudo do miocárdio e pericardite (6,11-19).

Estudos avaliando à fisiopatologia da COVID-19 pediátrica são ainda escassos. Uma das possíveis razões para crianças escolares e adolescentes apresentarem menores prevalências desta doença infecto contagiosa é a menor expressão do receptor da enzima conversora da angiotensina 2 (ECA-2) (4). O gene *ECA-2* expressa o RNA mensageiro estimulando a produção da ECA-2. Esta proteína funciona como um co-receptor fundamental para entrada do coronavírus nas células humanas, particularmente no pulmão, coração e intestino. Uma meta-análise evidenciou que algumas doenças crônicas podem mudar o programa epigenético, com aumento da expressão do gene *ECA-2*, favorecendo a infecção do coronavírus nas células pulmonares (20). Além disto, pacientes graves com COVID-19, particularmente com SIM-P, podem ter aumento das citocinas pró-inflamatórias, destacando-se: anti-TNF alfa, IL-1, IL-2, IL-6, IL-7 e fator estimulador de colônias de granulócitos (2,3,21).

Um fato relevante é que crianças escolares e adolescentes sobreviventes com a COVID-19 podem ter agressões isoladas ou concomitantes de vários órgãos e sistemas, tornando esta doença

infecciosa aguda como uma potencial condição crônica, podendo impactar prospectivamente a saúde física e mental da população pediátrica (2).

Adicionais estudos longitudinais, relevantes e originais serão necessários nos pacientes pediátricos com a COVID-19 tratados no HC-FMUSP, destacando-se: avaliações clínicas dos órgãos e sistemas (particularmente cardíaca, pulmonar, tireoide e pâncreas); avaliações do crescimento e da puberdade; estudos imunológicos (imunocompetência, incluindo avaliação do timo) e genéticos; impacto da COVID-19 na qualidade de vida relacionada à saúde (QVRS), saúde psíquica e física; composição corporal e metabolismo ósseo, assim como intervenções prospectivas, particularmente nos pacientes sobreviventes com SIM-P.

Recentemente, estudos indicam que a ECA-2 é também altamente expressa no pâncreas exócrino e nas ilhotas de Langerhans. Acometimento exócrino, com amilase e/ou lipase séricas elevadas ocorrem em 1-2% e 17% dos pacientes com a COVID-19, respectivamente. O SARS-CoV-2 pode agredir as células β pancreáticas e com possibilidade de induzir diabetes mellitus tipo 1 (22,23). Além disto, pacientes adultos com a COVID-19 podem apresentar reduções de hormônios tireoidianos (T3 e T4). Estudos em autopsia mostraram uma importante destruição de células foliculares e parafoliculares da tireoide, assim como este vírus poderia induzir autoimunidade órgão-especifica, como tireoidite autoimune. Um outro aspecto interessante é que os tecidos hipotalâmicos e hipofisários expressam ECA-2 e podem teoricamente ser alvos virais, podendo determinar hipofisite, lesão hipotalâmica e evolutivamente disfunção hipotálamo-hipófise. Estes aspectos podem determinar retardo no crescimento e puberdade atrasada em crianças escolares e adolescentes sobreviventes da COVID-19 (23).

Um outro aspecto relevante é que embora existam relatos de complicações neurológicas em indivíduos infectados pelo SARS-CoV-2, que ocorrem em 5% de adultos e 15% de crianças escolares e adolescentes, não há estudos avaliando o impacto prospectivo nas saúdes física e mental. A invasão do sistema nervoso central pode se dar por diferentes formas, como transferência transsináptica por neurônios infectados, entrada através do nervo olfatório, infecção do endotélio vascular ou migração de leucócitos através da barreira sangue-cérebro. Além do efeito direto do vírus, processos inflamatórios graves, que acometem crianças infectadas pelo vírus, também podem ter efeitos neuropsiquiátricos deletérios. Complicações neurológicas podem refletir efeitos neurotróficos no vírus, que pode invadir o cérebro e afetar o sistema renina-angiotensia (relevante para neuroproteção e cognição) e interagir com sistemas noradrenérgicos e dopaminérgicos

(24,25). De fato, crianças escolares e adolescentes sobreviventes com a COVID-19 podem ter impacto físico e da QVRS relacionado a condição crônica (como fraqueza muscular, alterações da marcha com disautonomia, sedentarismo, obesidade/sobrepeso), assim como impacto na saúde mental (vivência do luto de familiares, problemas de relacionamento com pais e familiares, disfunções sexuais, violência, uso abusivo de álcool e drogas ilícitas, transtornos psicológicos/psiquiátricos, assim como estresse pelo impacto financeiro e educacional causado pela doença).

Polimorfismos genéticos também tem sido alvo de estudos para tentar elucidar as diferenças interindividuais em relação à plasticidade neuronal e a sua relação com a recuperação motora e funcional. Variantes no gene BDNF, com destaque para o polimorfismo Val66Met (rs6265) tem sido relacionada com redução significante nos mecanismos de atualização ou reconsolidação da memória em indivíduos portadores do genótipo homozigoto mutado, quando comparados ao grupo de indivíduos homozigotos selvagem, já que a molécula BDNF teria influência sobre os processos de aprendizado e memória, que são muito importantes no processo de reabilitação. Dessa maneira, o papel desse polimorfismo na plasticidade cerebral e sua relação com a reabilitação de pacientes em diferentes condições aparentemente deve ser melhor explorada.

Aproximadamente 50% dos pacientes adultos após recuperação da infecção pelo SARS-CoV-2 têm apresentado fadiga, inflamação crônica e redução de aspectos da QVRS (26), podendo impactar a marcha, sistema nervoso autônomo, composição corporal, metabolismo ósseo e mesmo a realização de atividade física. Além disto, o isolamento social durante a pandemia de COVID-19 pode aumentar a inatividade física crônica e consequente risco de condições cardiovasculares crônicas (27). No entanto, pesquisas avaliando marcha, polimorfismos genéticos na plasticidade cerebral impactando na reabilitação, composição corporal, metabolismo ósseo, nível de atividade física, consumo alimentar e estudo de intervenção com atividade física ainda não foram realizados em crianças escolares e adolescentes que se recuperaram da COVID-19.

Justificativas

Crianças escolares e adolescentes sobreviventes com a COVID-19 podem ter inflamação persistente, curso crônico da COVID-19, com agressões isoladas ou concomitantes de vários órgãos e sistemas, tornando esta doença como uma potencial condição crônica, impactando QVRS, saúde física e mental.

Além disto, a COVID-19 pediátrica pode induzir autoimunidade (com possibilidade de tireoidite autoimune e diabetes mellitus tipo I), atraso do crescimento linear e do desenvolvimento puberal, imunodeficiência secundária e apresentar polimorfismos genéticos na plasticidade cerebral, impactando na reabilitação.

Tendo em vista que crianças escolares e adolescentes com a COVID-19 devam apresentar fraqueza muscular, disautonomia, astenia, desnutrição e sedentarismo, é fundamental que se desenvolvam intervenções seguras e eficazes de manutenção de níveis adequados de atividade física e que possam ser implementados em larga escala.

Entretanto, até o presente momento não há estudos longitudinais sistematizados que avaliaram todos estes aspectos em uma população pediátrica que sobreviveu a COVID-19, particularmente com condições crônicas e que foram hospitalizados em um serviço terciário.

Hipóteses

Crianças escolares e adolescentes sobreviventes com a COVID-19 poderão ter inflamação persistente, curso crônico da COVID-19, com agressões isoladas ou concomitantes de vários órgãos e sistemas, tornando esta doença como uma potencial condição crônica, impactando QVRS, saúde física e mental.

Objetivos

1) Avaliar possíveis agressões evolutivas aos órgãos (coração, timo, pâncreas e tireoide) em crianças escolares e adolescentes que se recuperaram da COVID-19 *versus* indivíduos que não apresentaram infecção pelo SARS-CoV-2.
2) Analisar impacto no crescimento e puberdade, na marcha, assim como imunodeficiência secundária e polimorfismos genéticos na plasticidade cerebral em crianças escolares e adolescentes sobreviventes da COVID-19 comparados aos pacientes que não apresentaram esta condição.
3) Verificar impacto nos aspectos da QVRS, saúde física e mental em crianças escolares e adolescentes que se recuperaram da COVID-19 *versus* indivíduos que não apresentaram infecção pelo SARS-CoV-2.

4) Estudar capacidade física, nível de atividade física e consumo alimentar, assim como avaliar a segurança e eficácia em um ensaio clínico randomizado de um programa de treinamento físico aeróbio em pacientes pediátricos sobreviventes da COVID-19.

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APPENDIX A – Ethics committee approval

USP - HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO - HCFMUSP

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Estudos prospectivos nas crianças escolares e nos adolescentes com a COVID-19 tratados no HCFMUSP

Pesquisador: Clóvis Artur Almeida da Silva

Área Temática:

Versão: 4

CAAE: 37460620.8.0000.0068

Instituição Proponente: Hospital das Clínicas da Faculdade de Medicina da USP

Patrocinador Principal: FUNDACAO DE AMPARO A PESQUISA DO ESTADO DE SAO PAULO

DADOS DO PARECER

Número do Parecer: 4.559.252

Apresentação do Projeto:

Nova documentação foi encaminhada

Objetivo da Pesquisa:

Trata-se de resposta ao Parecer anterior, informando que as pendências foram corrigidas

Avaliação dos Riscos e Benefícios:

Sem alterações

Comentários e Considerações sobre a Pesquisa:

O TCLE de pais e os dois Termos de assentimento ajustados para idade foram aprimorados

Considerações sobre os Termos de apresentação obrigatória:

Vide acima

Recomendações:

Não há

Conclusões ou Pendências e Lista de Inadequações:

Não há pendências

Considerações Finais a critério do CEP:

Endereço: Rua Ovídio Pires de Campos, 225 5º andar
Bairro: Cerqueira Cesar **CEP:** 05.403-010
UF: SP **Município:** SAO PAULO
Telefone: (11)2661-7585 **Fax:** (11)2661-7585 **E-mail:** cappesq.adm@hc.fm.usp.br

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1687858_E2.pdf	18/02/2021 11:15:36		Aceito
Declaração de Pesquisadores	resposta_parecer.PDF	18/02/2021 11:14:23	Juliana Caires de Oliveira Achili Ferreira	Aceito
Declaração de Pesquisadores	resposta_parecer.doc	18/02/2021 11:14:16	Juliana Caires de Oliveira Achili Ferreira	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_COVID_19_Emenda_final.docx	18/02/2021 11:13:22	Juliana Caires de Oliveira Achili Ferreira	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Assentimento_14_18_anos_Emenda.docx	18/02/2021 11:12:50	Juliana Caires de Oliveira Achili Ferreira	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Assentimento_9_13_anos_Emenda.docx	18/02/2021 11:12:26	Juliana Caires de Oliveira Achili Ferreira	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_pais_Emenda.docx	18/02/2021 11:09:35	Juliana Caires de Oliveira Achili Ferreira	Aceito
Declaração de Pesquisadores	FORMULARIO_Emenda1.doc	09/01/2021 11:02:33	Juliana Caires de Oliveira Achili Ferreira	Aceito
Declaração de Pesquisadores	FORMULARIO_Emenda1.PDF	09/01/2021 11:01:43	Juliana Caires de Oliveira Achili Ferreira	Aceito
Declaração de Pesquisadores	carta_emenda.PDF	09/01/2021 11:01:09	Juliana Caires de Oliveira Achili Ferreira	Aceito
Declaração de Pesquisadores	carta_emenda.doc	09/01/2021 11:00:59	Juliana Caires de Oliveira Achili Ferreira	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_pais_Emenda1.docx	09/01/2021 11:00:52	Juliana Caires de Oliveira Achili Ferreira	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Assentimento_14_18_anos_Emenda1.doc	09/01/2021 11:00:46	Juliana Caires de Oliveira Achili Ferreira	Aceito
TCLE / Termos de	Assentimento_9_13_anos_Emenda1.	09/01/2021	Juliana Caires de	Aceito

Cronograma	cronograma.pdf	11:06:37	Almeida da Silva	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

SAO PAULO, 25 de Fevereiro de 2021

Assinado por:
ALFREDO JOSE MANSUR
(Coordenador(a))

APPENDIX B – Detalhamento do programa de treinamento físico à distância.

PROGRAMA DE TREINAMENTO FÍSICO			
Observações importantes: 1. Priorizar: Prescrição das séries, o tempo de execução e as repetições de cada exercício. 2. Adaptações: Realizar as adequações necessárias de acordo com as características de cada doença e as dificuldades relatadas pelos pacientes. 3. Objetivo: Promover a progressão dos pacientes nos exercícios em cada nível, com adaptações realizadas ao longo do treinamento. 4. Cuidados especiais: o Pacientes com AIJ e comprometimentos articulares devem evitar exercícios pliométricos e dinâmicos. o Pacientes em uso de corticosteroides acima de 7,5 mg/kg devem evitar flexões de tronco. o Agachamento completo é contraindicado para pacientes com AIJ, dependendo do grau de comprometimento e estágio da doença. 5. Monitoramento: O grupo controle deve ser contatado a cada 15 dias.			
NÍVEL 1			
AQUECIMENTO 3X30 segundos - descanso entre as séries 2 minutos **PSE: 1° QUINZENA 4-5; 2° QUINZENA 5-6**			
1. Corrida no lugar	**2. Elevação de joelho**	**3. Polichinelo**	**4. Elevação de calcanhar**
Pontos de performance	**Pontos de performance**	**Pontos de performance**	**Pontos de performance**
Coordenar braço com a perna oposta	Levar o joelho até a linha do quadril, para facilitar, instruir o paciente a colocar a mão na frente do seu quadril	Tentar bater a palma da mão sobre a cabeça. Dica: abre e fecha as pernas ao mesmo tempo	Coloque as mãos sobre o glúteo e tente tocar seu calcanhar. Primeiro, devemos ensinar pausadamente, para eles fixarem o movimento
Adaptações	**Adaptações**	**Adaptações**	**Adaptações**
Realizar o movimento com as mãos na parede	Tentar realizar o movimento levando o joelho até a linha do quadril	Abrir e fechar os pés, parado no lugar	Realizar o movimento sem que o calcanhar toque no glúteo
1. Ao invés de correr no lugar, trotar vai e volta ou de um lado para o outro	1.Pode realizar o movimento mais devagar, até que chegue em uma velocidade onde a PSE fique moderada	1.Adaptação avançada: intercalar com polisapato	1.Coordenar movimento do ombro com o das pernas

PARTE PRINCIPAL 3X12 repetições - descanso entre as séries 2 minutos **PSE: 1° QUINZENA 4-5; 2° QUINZENA 5-6**				
1. Meio agachamento	**2. Abdominal curtinha**	**3. Flexão de braços com joelho**	**4. Avanço parado**	**5. Prancha frontal**

Pontos de performance	Pontos de performance	Pontos de performance	Pontos de performance	Pontos de performance
Pés afastados na largura do ombro, totalmente apoiados no chão, curvatura lombar mantida em C, os braços podem estar apoiados nos ombros para facilitar. O movimento deve começar com o quadril	Os braços podem estar no peito ou atrás da cabeça. Realizar a retroversão do quadril no início do movimento, evitando a curvatura lombar. É importante que o abdômen esteja contraído a cada repetição. Ao realizar a elevação do tronco, soltar o ar. Coordenar respiração com o movimento	Braços afastados na linha do ombro, ao chegar nessa posição, ir afastando as mãos, levemente, até que o paciente se sinta numa posição confortável. Uma forma que gosto de ensinar é: a partir dessa posição, eu peço que eles se deitem no chão, levando a barriga e o corpo todo em direção ao chão. Ao levantar, eles devem fazer a força com os braços (peitoral, tríceps), não esquecer de contrair o abdômen para que eles não fiquem com a curvatura lombar acentuada na volta do movimento	Pés e joelhos apontados para frente. O afastamento lateral das pernas deve dar a estabilidade necessária para que o paciente não fique desequilibrado. O joelho da frente não deve ir muito à frente e o de trás deve quase tocar ao chão	Cotovelos na linha do ombro, abdômen para "dentro" em contração. Peça sempre para o paciente contrair todo o corpo, isso inclui, glúteos, coxa e abdômen. Cuidado com a curvatura lombar, não deve ficar com lordose protuberante
Adaptações	**Adaptações**	**Adaptações**	**Adaptações**	**Adaptações**
Adaptação: sentar e levantar de uma cadeira	Colocar os pés em algum apoio (ex. embaixo da cama ou sofá)	Realizar o movimento na parede ou no sofá.	No início, eles podem realizar o movimento se apoiando nas laterais, como paredes etc., até desenvolver a coordenação necessária	Prancha com os braços estendidos, ao invés do cotovelo flexionado. Realizar o movimento sobre superfície como sofá, banco etc.
Objetivo: tirar a cadeira aos poucos até que faça um agachamento 90° graus	Adaptação avançada: abdominal com os pés elevados. O objetivo é tocar ambas as mãos nos pés	Realizar o movimento de joelhos com amplitude reduzida. O objetivo é que ele ganhe força e vá aumentando a amplitude conforme for se adaptando ao movimento	Movimento mais curtos, até chegar na amplitude desejada	Prancha com o cotovelo flexionado, mas com os joelhos no chão. Pode diminuir o tempo se conseguir manter a prancha sem os joelhos no chão

NÍVEL 2				
AQUECIMENTO 3X40 segundos - descanso entre as séries 1 min e 30 seg. PSE: 1° QUINZENA 5-6; 2° QUINZENA 6-7				
1. Tocar os pés	**2. Frankenstein**	**3. Saltos no lugar**	**4. Escalador**	
Pontos de performance	**Pontos de performance**	**Pontos de performance**	**Pontos de performance**	
Posição inicial: prancha com os cotovelos estendidos e afastados além da linha do ombro para dar mais estabilidade na hora do paciente rotacionar o tronco). De maneira lúdica, eles devem tentar tocar a ponta do pé com a mão oposta	Exercício coordenativo, onde o paciente deverá levar seu pé direito em encontro ao seu braço esquerdo. Para iniciar, podemos pedir para que eles imitem uma múmia ou algo do tipo, com os braços a frente e tentando levar a perna de encontro com o braço. Depois, falar para eles fazer isso de maneira contrária, ex.: Tente levar sua perna direita de encontro com seu braço esquerdo, como se fosse o Frankenstein (pode até pedir para eles fecharem os olhos para trabalhar propriocepção também)	A posição inicial é semelhante ao meio agachamento. Quanto maior for a fase excêntrica do movimento, mais difícil e intenso ficará. Aconselho a começarem em um meio agachamento e descer na altura que acharem	Posição inicial: prancha com os cotovelos estendidos. O objetivo é levar o joelho em direção ao cotovelo. Prestar atenção na posição da lombar e escapulas (não deixar elas entrarem em abdução extrema enquanto estiver na prancha)	
Adaptações	**Adaptações**	**Adaptações**	**Adaptações**	
1. Tocar a ponta do pé com a mesma mão. 2. Leg raise	1. Sem adaptações, acredito que seja um movimento simples e que todos conseguirão executar	1. Saltar e tentar manter os pés no mesmo lugar. Iniciar o movimento com uma leve semiflexionada nos joelhos. Não precisa necessariamente agachar. 2. Flexão plantar	1. Aumentar a velocidade aos poucos. 2. Utilizar cadeira	
2. Diminuição do tempo de execução, realizando o exercício proposto		2. Realizar o movimento com pouca flexão/extensão do joelho		
PARTE PRINCIPAL 4X10 descanso entre as séries de 1 min e 30 segs. PSE: 1° QUINZENA 5-6; 2° QUINZENA 6-7				
1. Agachamento completo	**2. Tríceps na cadeira**	**3. Prancha lateral**	**4. Subindo cadeira**	**5. Abdominal remador**
Pontos de performance	**Pontos de performance**	**Pontos de performance**	**Pontos de performance**	**Pontos de performance**

Posição inicial: Em pé, com as pernas paralelas e afastadas na linha do quadril e mãos entrelaçadas à frente do corpo com os cotovelos flexionados. Flexione os joelhos até formar um ângulo de 90 graus, empurrando o quadril para trás. Atenção: joelhos não devem ultrapassar a linha das pontas dos pés	Posição inicial: Sente-se em uma cadeira e posicione as palmas das mãos no apoio, afasta-se em uma distância um pouco maior do que a largura dos ombros; estenda as pernas e mantenha os calcanhares apoiados no solo; Mova o corpo para a frente até tirar os glúteos da cadeira; Flexione os cotovelos em aproximadamente 90º, descendo o corpo e apoiando seu peso nos braços; Retorne a posição inicial sem estender os cotovelos	Posição inicial: Fique na posição lateral; apoie apenas o cotovelo ao solo e os pés, mantenha a cabeça em uma posição neutra, a coluna reta. 2. Colocar o pé na frente	Posição inicial: Fique parado em frente a cadeira, você irá fazer o movimento de subir e descer um degrau (subindo uma perna e em seguida a outra). Atenção, antes de iniciar o movimento deixe a cadeira apoiada em uma parede para não ter risco de derrubá-la	Posição inicial: deitado no solo com braços e pernas estendidos. Após se posicionar, basta flexionar as pernas ao mesmo tempo que contrai o abdômen em direção aos joelhos, abraçando-os. Atenção, mantenha sempre o abdômen contraído, e coordene a respiração com o movimento
Adaptações	**Adaptações**	**Adaptações**	**Adaptações**	**Adaptações**
1.Caso esteja muito difícil, faz em uma menor amplitude e aos poucos aumentá-la	1.Faça esse movimento no solo, o que irá mudar é que os braços estarão estendidos e o movimento será feito com o quadril. 1. na parede 2. na cadeira com amplitude menor	1.Realizar o movimento apoiando os joelhos no chão	1.Aumentar a velocidade aos poucos. 2. Marcação do passo na cadeira	1.Aumentar a velocidade aos poucos
2.Para dificultar um pouco ao subir, suba na ponta dos pés				

NÍVEL 3				
AQUECIMENTO 3X45 segundos – intervalo de descanso entre as séries: 1 minuto PSE: 1° QUINZENA 6-7; 2° QUINZENA 8-9				
1. Alongamento de quadril	**2.Saltos laterais**	**3. Polisapato**	**4. Salto em distância**	
Pontos de performance	**Pontos de performance**	**Pontos de performance**	**Pontos de performance**	
Iniciar a posição em decúbito frontal, após, colocar o joelho próximo ao peito e puxar. Pode realizar o movimento com as pernas esticadas	Iniciar o movimento com os pés juntos, demarcar um ponto no chão, pode ser um chinelo, faça com que o paciente tente saltar esse objeto para ficar de uma maneira mais lúdica e de fácil compreensão	Tentar bater a palma da mão sobre a cabeça. Dica: abre e fecha as pernas ao mesmo tempo	Iniciar o movimento com os pés juntos, movimentando os braços para trás, demarcar um ponto no chão com uma distância que o paciente consiga saltar o mais longe possível da posição inicial	
Adaptações	**Adaptações**	**Adaptações**	**Adaptações**	
Puxar as pernas com movimentos dinâmicos Opção 2*: spider man*	1.Corrida lateral 2. Passos laterais (sem salto)	Aumentar a velocidade aos poucos	Passos em distância	
PARTE PRINCIPAL 4X12 repetições – descanso entre as séries: 1 minuto PSE: 1° QUINZENA 6-7; 2° QUINZENA 8-9				
1. Cadeirinha (máximo)	**2. Avanço alternado**	**3. Abdominal elevando as pernas**	**4. Flexão + escalador**	**5. Superman**
Pontos de performance	**Pontos de performance**	**Pontos de performance**	**Pontos de performance**	**Pontos de performance**
Posição inicial: Com as costas apoiadas na parede, separe as pernas na largura dos ombros. Flexione os joelhos e desça em direção ao chão, chegando aos 90°. As costas permanecerão eretas e encostadas na parede, o objetivo é sustentar nessa posição	Posição inicial: Em pé com os pés afastados na largura dos ombros; os braços podem estar apoiados nos ombros ou com as mãos na cintura. Dê um passo para a frente e flexione o joelho até que a coxa da perna que avanço esteja paralela com o chão	Posição inicial: Apoiado com a coluna no solo, as mãos precisam estar retas e posicionadas abaixo do seu quadril. Após se ajustar, basta erguer as pernas lentamente, mantendo elas o mais retas possível	Idem a descrição das anteriores, porém acrescentar a flexão de quadril junto ao movimento de flexão de braço. A cada flexão de braço, 2 movimentos de flexão de quadril	Posição inicial: Apoiado com a coluna no solo, as mãos precisam estar retas e posicionadas abaixo do seu quadril. Semelhante ao abdominal anterior, mas nesse movimento eleve as pernas e alterne-as no ar

	Retorne à posição inicial e repita, usando a outra perna			
Adaptações	**Adaptações**	**Adaptações**	**Adaptações**	**Adaptações**
Flexione o joelho até onde sentir-se confortável, mesmo que seja em um ângulo inferior a 90º	Realizar uma perna de cada vez, lentamente	Flexione as pernas e faça o movimento com elas flexionadas	Movimento de joelhos no chão	Unilateral isométrico ou superman dinâmico
Para dificultar um pouco, tire o calcanhar do solo e mantenha o movimento na ponta dos pés	Para dificultar um pouco, o movimento pode ser realizado de forma mais dinâmica, saltando-se			