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Multisystem Inflammatory Syndrome in Children:

longitudinal body composition assessment, inflammatory markers, and fatty acids profile

MED/38- MED/49

Doctoral Dissertation :

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Contents

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Abstract

The Sars-Cov 2 pandemic mainly affected the adult population. However, a small portion of children were affected by Multisystem Inflammatory Syndrome in Children (MIS-C). The severity of the syndrome required patients to be hospitalised and, in some cases, admitted to pediatric intensive care units. A high risk of malnutrition was detected in children hospitalised for long periods. In addition, omega-3 fatty acids (ω-3 FA) were used in conjunction with drug therapy in adults hospitalised for Covid-19 to improve the inflammatory response.

The aim of this work was to assess the risk of malnutrition in the acute phase and body composition over time in a cohort of children diagnosed with MIS-C. For all the recruited children, inflammatory markers, metabolic parameters, and fatty acid levels were assessed both in the acute phase and six months after discharge, alongside anthropometric assessment. In half of the patients recruited, three months of docosahexaenoic acid (DHA) supplementation was also recommended. We observed a change in body composition in the acute phase, with a reduction in fat-free mass. In contrast, in the long term we detected a gain in fat mass, probably due to the limited spontaneous and structured physical activity on the part of the children.

The fatty acid levels detected in the acute phase were lower than in the healthy population, while the glucose profile was altered. At three and six months after discharge, the ω-3 FA values were higher than the acute phase, particularly in the group that received supplementation. The correlation between ω-3 FA levels and inflammatory indices at six months after discharge in the supplemented group, showed a positive correlation between ω-3 eicosapentaenoic acid (EPA) and erythrocyte sedimentation rate (ESR)and d-dimer, as well as a positive correlation between DHA and ferritin. Overall, the MIS-C children had a favourable disease course. However, nutritional status and loss of body weight should always be monitored in this type of disease during hospitalisation. In the long term,

adherence to a balanced diet should be advised along with the resumption of spontaneous and planned physical activity. At the same time, ω-3 FA supplementation may facilitate optimal recovery. As a future perspective, the effectiveness of ω-3 supplementation in the acute phase and later, in seriously ill paediatric patients should be explored.

Chapter 1

Introduction

1.1 Definition, epidemiology, and geographical distribution of Sars-Cov2

The new 2019 coronavirus (CoV), later named 'severe acute respiratory syndrome by Coronavirus-2 (SARS- CoV- 2)' by the International Committee on Taxonomy of Viruses, belongs to the beta-CoV genus. Beta-CoV also includes the Severe Acute Respiratory Syndrome virus (SARS-CoV) and Middle East Respiratory Syndrome virus (MERS-CoV) . [1] At the end of December 2019, several health centres in Wuhan, in China's Hubei province, reported clusters of patients with pneumonia of an unknown cause [2], which after about one month had been reported in all 34 provinces of China [3]. On 30 January, the WHO declared the outbreak of the new coronavirus an international public health emergency, and on 11 March 2020, it officially designated the global epidemic of COVID-19 as a pandemic [3]. Figure 1 shows the time sequence of the most relevant events since the start of the pandemic.

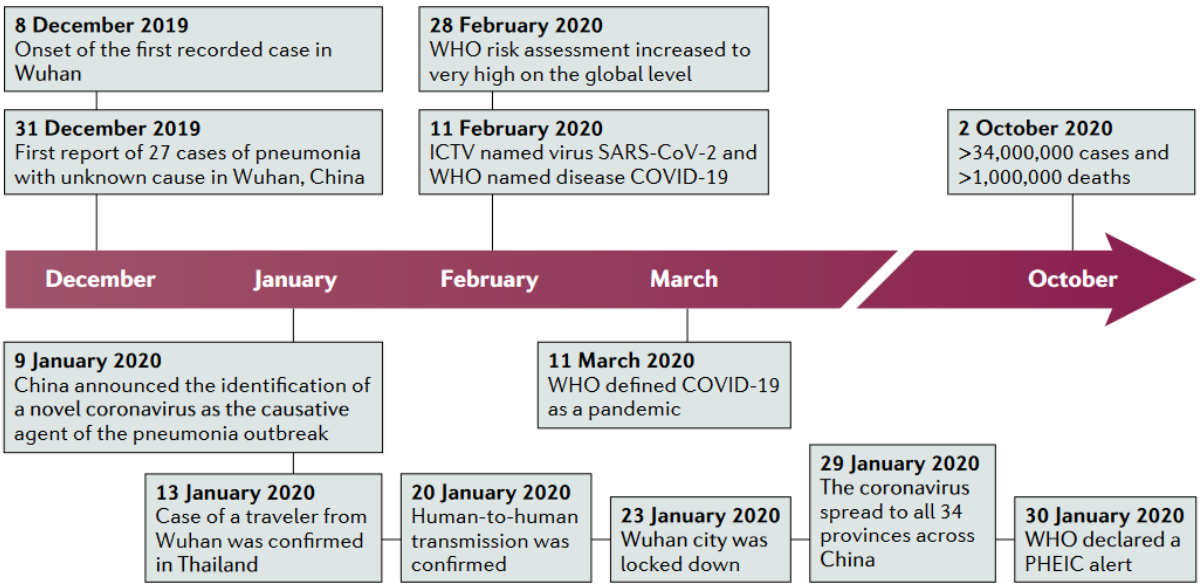

Fig. 1 – Chronology of the main events of the COVID-19 outbreak.

Abbreviations: ICTV, International Committee on Taxonomy of Viruses; PHEIC, public health emergency of international concern; [3].

In September 2020, there was also a substantial increase in new cases and deaths in the eastern Mediterranean region and in in the USA. Figures 2 and 3 show the geographical distribution of the 20 countries most infected by the virus and the deaths in these countries[1].

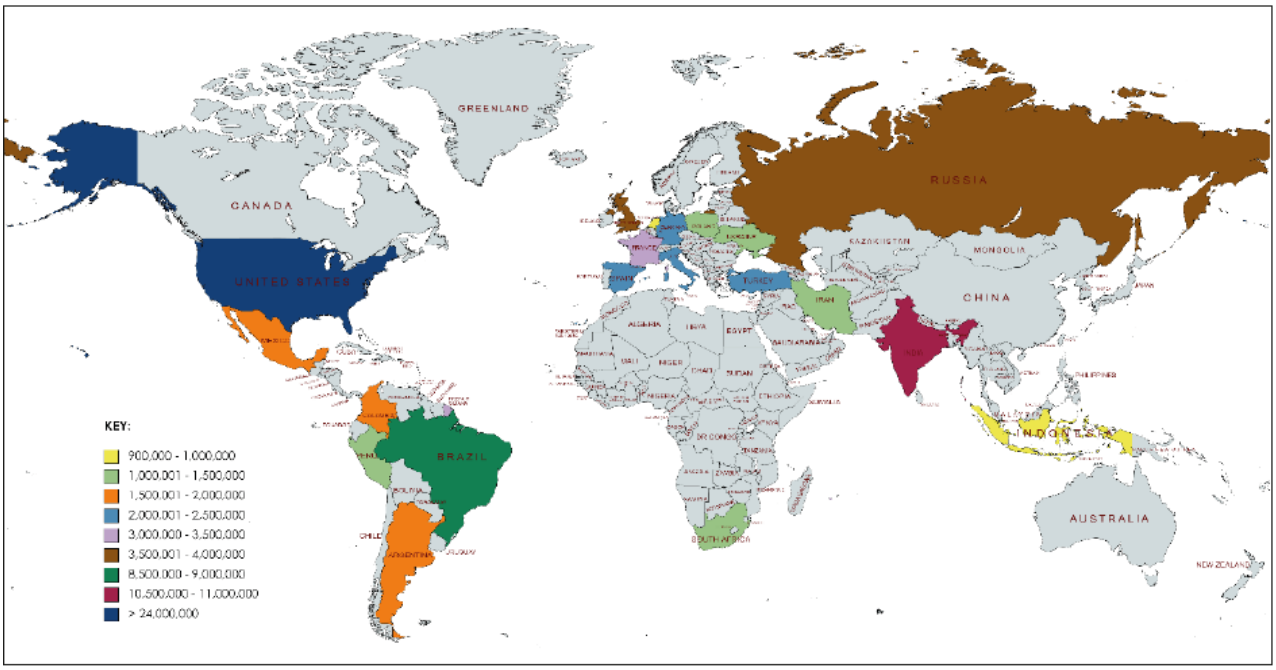

Fig. 2 - The 20 countries most affected by COVID-19 [1]

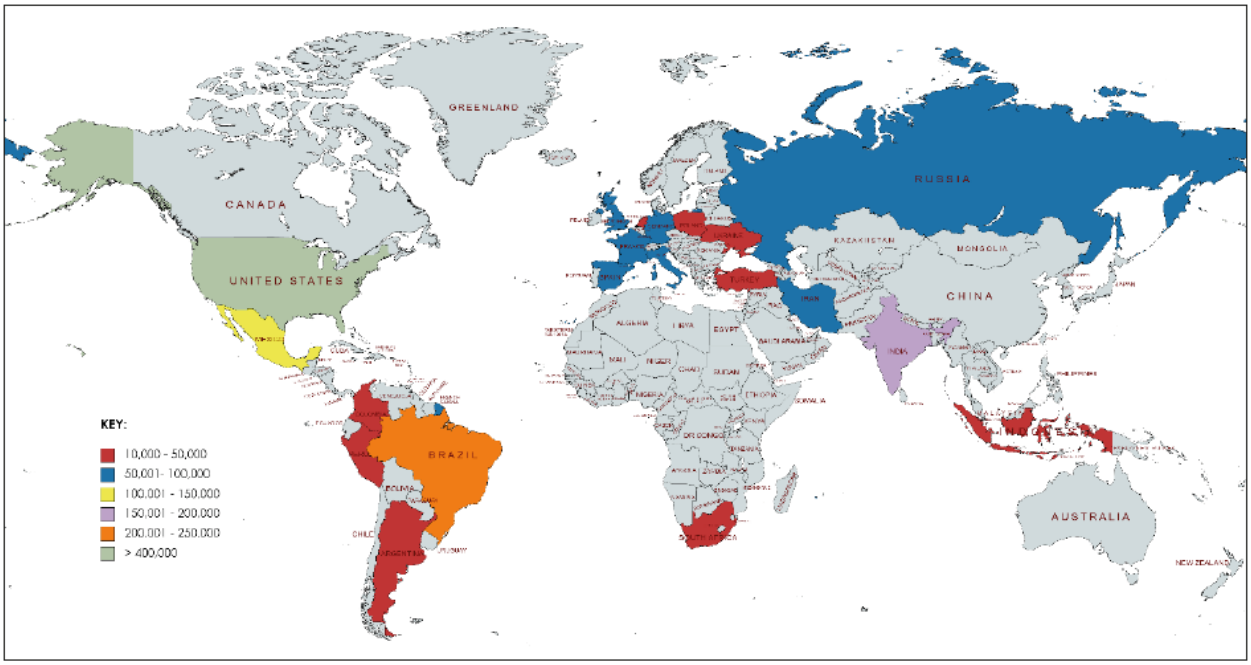

Fig. 3 – Deaths from COVID-19 in the 20 countries most affected [1]

Clinical manifestation in adults and children

The clinical manifestations of COVID-19 vary according to age: elderly men (>60 years) with comorbidities are more likely to develop severe respiratory disease requiring hospitalisation [4,5].On the other hand, data initially indicated that children under the age of 18 were less likely to develop the disease than adults [6,7]. During the initial phase of the pandemic, it was in fact thought that children were 'immune' or largely spared the comorbidities and mortality associated with COVID-19, however, several studies have since reported the development of severe or even critical complications among children with COVID-19 [6,7]. Figure 4 summarises the clinical features of the disease in the different age groups.

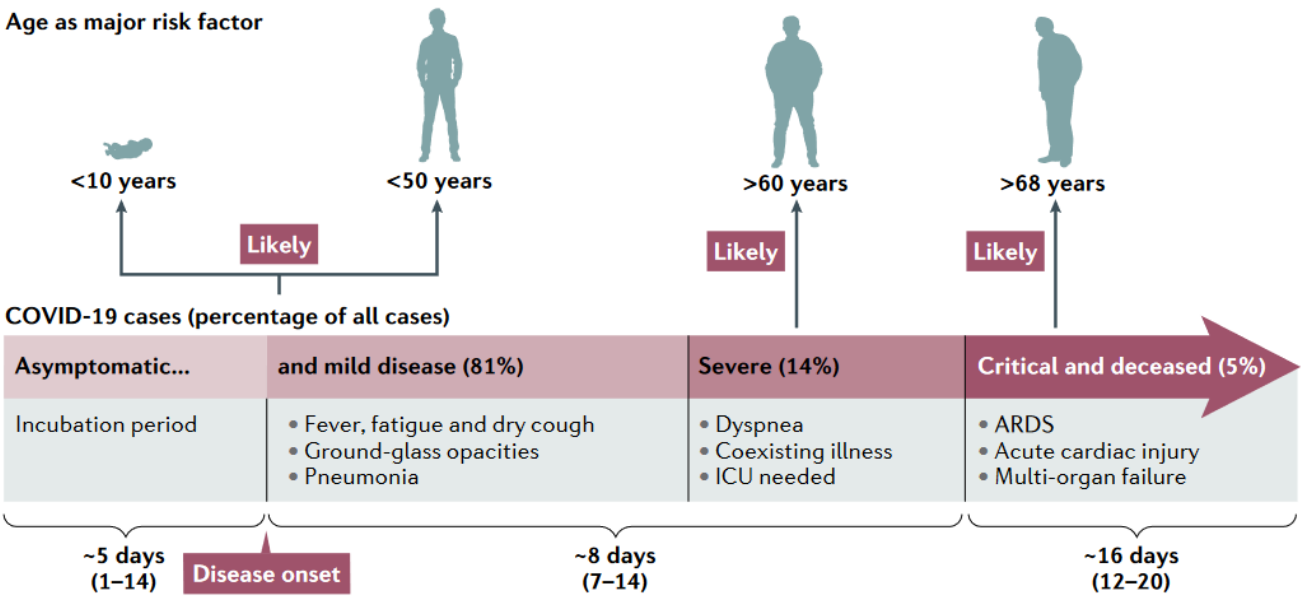

Fig. 4 - Clinical features of COVID-19. *Typical symptoms of the disease are fever, dry cough, fatigue and, in severe cases, dyspnoea. Many infections, particularly in children and young adults, are asymptomatic, whereas the elderly and/or persons with co-morbidity are at increased risk of severe illness, respiratory failure, and death. The incubation period is about five days, severe illness usually develops about eight days after the onset of symptoms. Critical illness and death occur at about 16 days. ARDS = acute respiratory distress syndrome; PICU = intensive care unit.* [3]

1.2 Multisystem Inflammatory Syndrome in Children (MIS-C)

In April 2020, during the peak of the pandemic in Europe, data were published regarding British children presenting with hyper-inflammatory shock, with features similar to Kawasaki disease (KD) and toxic shock syndrome (TSS). The Royal College of Paediatrics and Child Health initially defined this acute condition as paediatric multisystem inflammatory syndrome temporally associated with COVID-19 (PIMSTS)[8]. With the emergence of more cases worldwide, the disease was renamed 'Multisystem Inflammatory Syndrome in Children' (MIS-C) by the US Centres for Disease Control and Prevention (CDC) and the World Health Organisation (WHO). This syndrome appears to have a probable relationship with SARS-CoV-2 infection [9]. Table 1 shows the diagnostic criteria for MIS-C developed by the CDC [10].

Table 1: Criteria for the diagnosis of MIS-C established by the CDC [10]

CRITERIA FOR THE DIAGNOSIS OF MIS-C ACCORDING TO THE CDC	
Age	<21 years
Fever	≥ 38.0° C for ≥ 24 hours
Symptoms	Severe illness (hospitalisation) ≥ 2 organs involved
Inflammation	Increase in more than one of the following indices of inflammation: - PCR - ESR - Fibrinogen - Procalcitonin - D-dimer - Ferritin - LDH - IL-6 - Neutrophilia - Lymphopenia - Hypoalbuminemia
Sars-Cov-2	Current or recent positivity for: - Molecular test - Serology - Antigenic test /Exposure to COVID-19 in the previous 4 weeks
Exclusion	No alternative diagnosis

According to the diagnostic criteria developed by the CDC in May 2020, a subject with MIS-C is under 21 years of age with a history of at least 24 hours of fever of 38.0 °C, presenting with a severe illness requiring hospitalisation and two or more organ systems affected (e.g. cardiac, renal, respiratory, haematological, gastrointestinal, dermatological, neurological). The subject must also show evidence of inflammation with high levels of C-reactive protein (CRP), ESR, fibrinogen, procalcitonin, D-dimer, ferritin, LDH or IL-6; reduced lymphocytes or elevated neutrophils, reduced albumin and lastly, evidence of SARS-CoV-2 infection by a positive test for SARS CoV-2 by RT-PCR, serology or antigen test or exposure to COVID-19 in the four weeks preceding the onset of symptoms. The clinical features of MIS-C are similar to some paediatric rheumatological diseases, such as Kawasaki disease (KD), which affects small and medium-sized vessels. Other features are similar to those of macrophage activation syndrome (MAS). The diagnostic criteria for MIS-C differ in in terms of myocardial involvement, lymphopenia, neutrophilia, and increased ferritin levels in peripheral blood [11]. In MAS, the ESR (erythrocyte sedimentation rate) is low, whereas in MIS-C it is significantly elevated, and the cytokine storm associated with MAS is also not exactly the same as that found in inflammatory diseases that develop post-COVID-19 [11].

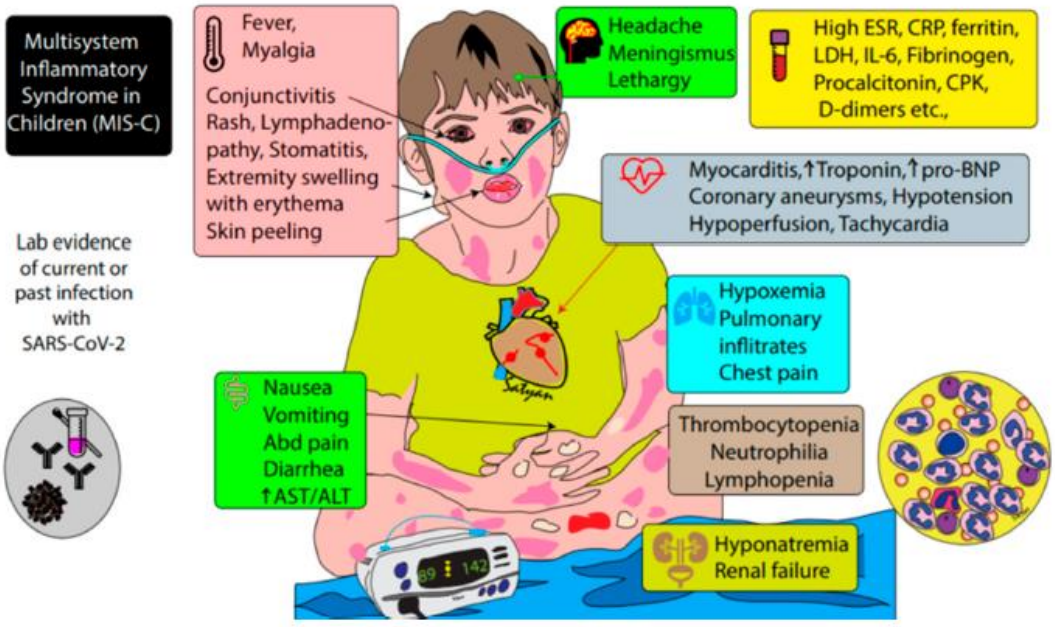

Fig. 5 CDC criteria for the diagnosis of MIS-C. *A combination of fever, inflammation, involvement of at least two organ systems and previous evidence of SARS-CoV-2- 2 infection are required to establish a diagnosis* [9] .

1.2.1 Mechanism of viral infection, transmission, and possible pathogenesis of MIS-C

The possible pathogenic mechanism proposed by Ochani et al.[1], regards the four structural proteins of the CoV, S, N, M and E, which allow the virus to gain access to the host cells. The S protein is strongly N-glycosylated, and the M protein is present as a dimer in the virion and maintains its shape. On the other hand, the E protein is a transmembrane protein with ion channel activity that plays an important role in viral pathogenesis as it promotes the assembly and release of the virus from the host cell. Binding by the S protein to its receptor enables the virus to enter human cells. The S protein directs cell-cell fusion between infected cells, leading to the formation of large multinucleated cells that are undetected by antibodies, thus enabling the virus to spread within an infected organism. SARS-CoV-2 utilises the angiotensin-converting enzyme-2 (ACE2) receptor to facilitate virus entry into target cells, resulting in down-regulation of these receptors and increased production of angiotensin-2 (AT2). Increased AT2 production potentially increases pulmonary vascular permeability and may cause lung injury. Approximately 83% of ACE2 receptors are expressed on the luminal surface of alveolar epithelial type II cells, making the lungs the primary reservoirs of viral invasion. However, the multi-organ dysfunction observed in patients could be attributed to the wide distribution of ACE2 receptors in extra-pulmonary tissues, including the heart, kidneys, endothelium and intestine [1].

When a virus infects an organism, innate and adaptive immune responses are activated through the first macrophage, which recognises the foreign agent and induces the presentation of CoV antigens to T cells[11]. This process leads to T-cell activation and differentiation, including the production of cytokines associated with different T-cell subpopulations, followed by the release of cytokines to amplify the immune response., In response to CoV infection, infected cells secrete large numbers of chemokines and

cytokines (IL-1, IL-6, IL-8, IL-21, TNF-β and MCP-1)[11] . These chemokines and cytokines, in turn, recruit lymphocytes and leukocytes to the site of infection and contribute to the cytokine storm. SARS-CoV-2 also causes viral sepsis, and the resulting initial immune response is an increase in pro-inflammatory cytokines and a severe immune crisis [11]. Figure 6 illustrates the main pathways through which the host organism recognises and responds to coronaviruses.

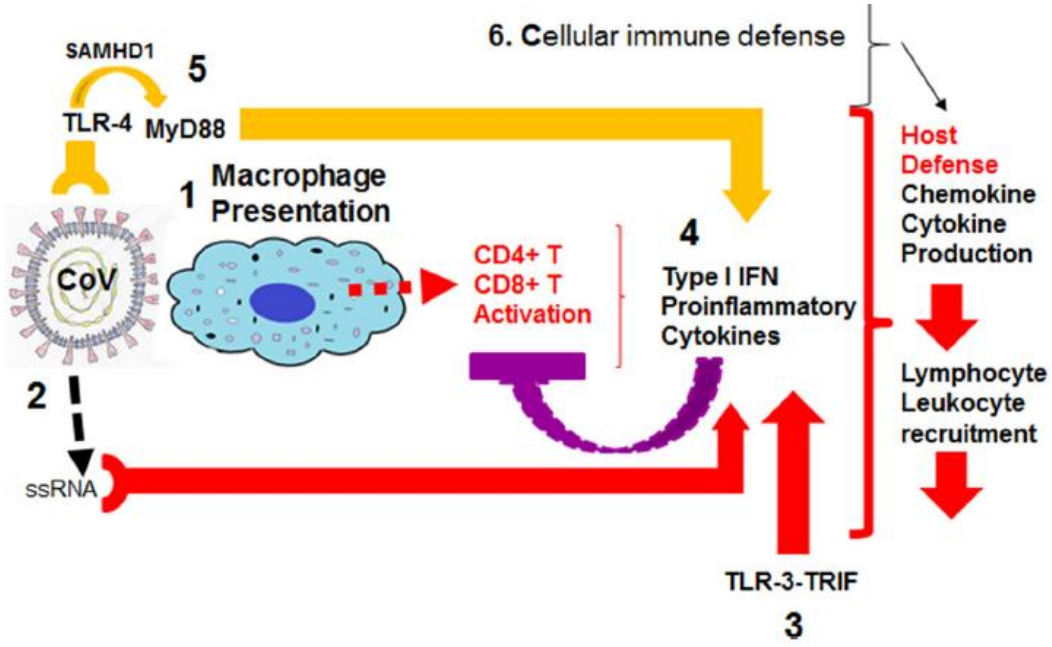

Fig. 6– Host immune defence against coronavirus infections [11].

After infection of a human host, horizontal transmission of SARS-CoV-2 occurs mainly through contact between humans: directly through respiratory droplets (droplets) or indirectly by touching contaminated surfaces[12]. With regard to vertical transmission, data indicate that there is a low probability of a mother transmitting the virus to her new-born [13]. An important factor for transmission is the spread of the virus by people with asymptomatic infection, which may account for 25% to 50% of new infections [1].

Epidemiological evidence points to SARS-CoV-2 infection as the likely cause of MIS-C, although causality has not yet been established with certainty [14] Most of the cases reported had positive serological tests for SARS-CoV-2, and less commonly, positive RT-PCR tests

from nasopharyngeal swabs, which suggests that this hyper-inflammatory syndrome may be post-infectious rather than related to an early acute infection (phase 1-early infection) [9].

In adults with severe respiratory failure due to SARS-CoV-2 infection, who typically present with clinical deterioration about one week after the onset of the disease, it is thought that it is the altered immune system that drives the disease manifestation, as opposed to direct cellular damage by the viral infection (phase II – pulmonary) [9] . Children appear to have less severe pulmonary manifestations than adults, possibly due to lower gene expression of the receptor for ACE-2, the target of SARS-CoV-2. It was therefore hypothesised that MIS-C is a delayed immunological phenomenon associated with inflammation (phase III - hyperinflammation) following symptomatic or asymptomatic COVID-19 infection.

This immune dysregulation is associated with the inflammatory syndrome in affected children. Direct infection with SARS-CoV-2 is less likely to play a role in MIS-C [9]. It has also been suggested that there may be a genetic susceptibility that favours the development of MIS-C. Studies on hospitalised adults with severe COVID-19 have identified deleterious genetic variants that impaired the type I interferon signalling pathway. In contrast, MIS-C is not associated with cardiopulmonary, autoimmune and/or immune or haematological diseases and its genetic basis is unknown. Previous studies have reported haploinsufficiency of the suppressor of cytokine signalling 1 (SOCS1), a negative regulator of type I and type II interferons, as a risk factor for the development of MIS-C. In a prospective study of 18 patients with MIS-C, 17% were found to have a genetic variant that impairs the negative regulation of interferon and inflammatory signalling [15]. Figure 7 shows the MIS-C pathogenesis mechanism.

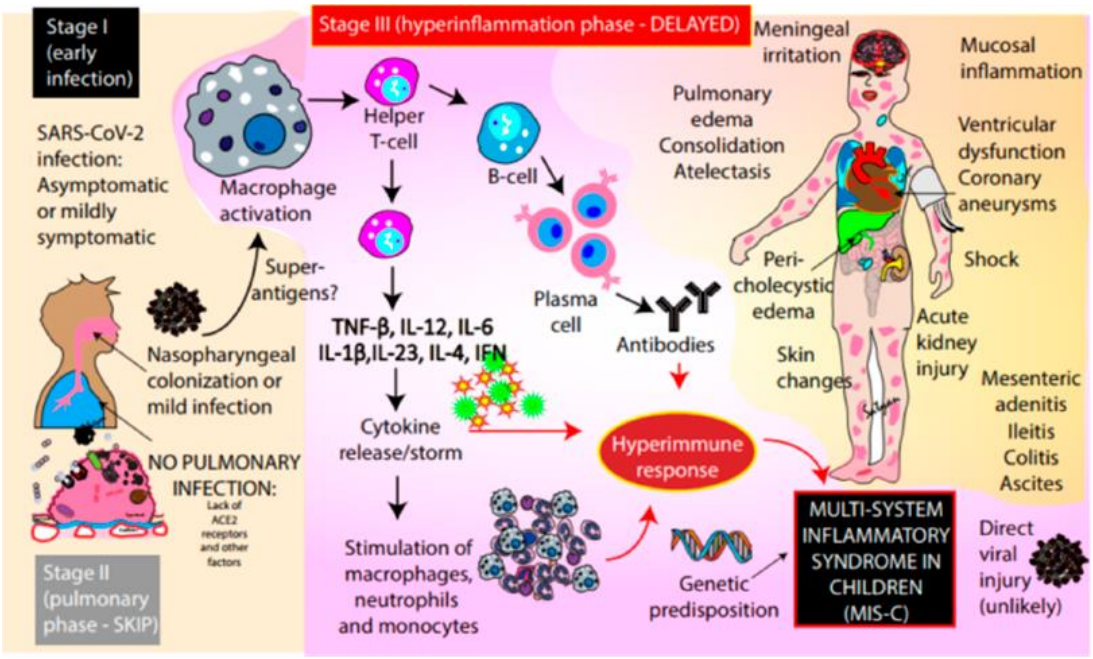

Fig. 7 –MIS-C Pathogenesis. *Early infection (phase I) with SARS-CoV-2 is probably asymptomatic or mildly symptomatic in children. The pulmonary phase (phase II) is severe in adults, but mild or absent in many children. Early infection appears to trigger the activation of macrophages followed by stimulation of T-helper cells. This in turn leads to cytokine release, stimulation of macrophages, neutrophils and monocytes, as well as the activation of B-cells and plasma cells with the production of antibodies leading to a hyperimmune response (phase III). Abbreviations: ACE2 - angiotensin-converting enzyme receptor 2; TNF - tumour necrosis factor-tumour necrosis factor; IL - interleukins.*[9]

1.2.2 Clinical manifestation in MIS-C

Several systematic reviews of data on the paediatric population in a case series in the United Kingdom [16], Italy [17], France and Switzerland [18,19], reported that children affected by this syndrome (n=70) were aged between 2 and 16 years. Most of the children had a fever for four days. The most common symptoms included gastrointestinal symptoms (59/70 = 84%), including vomiting, abdominal pain and/or diarrhoea; muco-cutaneous symptoms, similar to those of KD, including conjunctivitis and rash; and lastly neurological symptoms, including headache, irritability and encephalopathy. Several children developed hypotension (52/70 = 74%) and required admission to the intensive care unit, and some required mechanical ventilation. A minority of children (11/70 = 16%) underwent extracorporeal membrane oxygen support (ECMO) [9].

A systematic review and meta-analysis, which included 97 studies from 18 different countries with a total of 2275 patients under the age of 18 years (mean age 9 years), found that fever (100%), gastrointestinal pain (82%), and abdominal pain (68%) were the main symptoms reported in MIS-C patients. Cardiac symptoms (66%) prevailed over respiratory (39%) and neurological (28%) symptoms [8].

Another systematic review including studies published in the USA, France, UK, Italy, Spain and India between February and July 2020, characterised and evaluated cases of MIS-C in paediatric patients associated with coronavirus infection (SARS-CoV-2). The average duration, from symptom onset to hospital admission, was four days. Fever was the most common symptom, followed by gastrointestinal (GI) manifestations, which were found in 70% of the patients with symptoms such as viral gastroenteritis or inflammatory bowel disease accompanied by nausea, vomiting, diarrhoea, and abdominal pain. Cardiovascular symptoms were reported in 51% of patients. Hypotension was reported in 28% of patients. KD-like symptoms were observed in 36% of patients, of which a minority had features of classic/typical KD and the majority had atypical features. Central nervous system involvement was reported in 12 studies with 22% of children presenting with aseptic meningitis, headache or altered mental status. Only eight studies reported respiratory symptoms such as cough and congestion[20].

A retrospective review [21] published in February 2021, reported a case series of children who met the published definition of MIS-C and who were discharged or died between 1 March 2020 and 15 June 2020 from 33 European, Asian and American hospitals. In this study, 183 children with MIS-C were included: of these 109 (59.6 %) were male; 56 (30.6 %) were of Black ethnicity and 48 (26.2 %) were obese. Overall, 114 out of 183 (62.3%) had evidence of coronavirus infection. All of the children presented with fever, 117 out of 183 (63.9%) with gastrointestinal symptoms, and 79 out of 183 (43.2%) with shock and increased inflammation associated with Black ethnicity.

A study by Parri et. al, involved a cohort of 100 children in Italy under the age of 18 years with Covid-19 confirmed by molecular testing (nasal or nasopharyngeal swabs), evaluated

between 3 and 27 March 2020 in 17 paediatric emergency departments. The mean age of the children was 3.3 years. Exposure to SARS-CoV-2 from an unknown source or from a source outside the child's family accounted for 55% of the infection cases . A total of 12% of the children appeared to be ill and 54% had a temperature of at least 37.6°C. The most common symptoms were cough (in 44% of patients) and lack of appetite or difficulty in feeding (in 23%); the latter symptom occurred most often in children under 21 months of age. Fever, cough or shortness of breath occurred in 28 of the 54 patients with fever (52%) . A total of 4% of the children had oxygen saturation values below 95%; all these patients also had imaging evidence of lung involvement [7].

1.3 Sars-Cov 2 and comorbidity in paediatric patients

A systematic review and meta-analysis of the literature assessed effects of paediatric comorbidities on the severity of COVID-19 [22]. Forty-two studies were considered which included 275,661 children without comorbidities and 9,353 children with underlying comorbidities (obesity, chronic respiratory disease, cardiovascular disease, immune system disorders, metabolic disease, cancer, renal disease and gastrointestinal disorders). Among the 9,353 paediatric patients with SARS-CoV-2 infection and underlying comorbidities, 481 (5.1%) had a severe COVID-19 manifestation and/or were admitted to intensive care. In contrast, only 579 of 275,661 (0.21%) paediatric patients without comorbidities had a severe manifestation of COVID-19 [22]. The authors subsequently evaluated the potential impact of specific comorbidities on the risk of severe disease manifestation and found that, among children with severe COVID-19, 64 were obese, 26 had immune disorders, 19 had metabolic diseases, and 2 had gastrointestinal comorbidities. The authors analysed the relative contribution of childhood obesity to COVID-19 disease severity and the results indicated that childhood obesity probably increased the risk of severe COVID-19 [22].

A multicentre retrospective cohort study, conducted in eight medical centres, and including all patients under 18 years of age admitted to intensive care units as a direct consequence of COVID-19 disease, described the clinical characteristics of the children

admitted to intensive care units. A total of 88% of the children had comorbidities: 24% of the patients had an underlying neurological disease, 20% had haematological malignancies, 16% had congenital heart disease, and 8% were obese [23].

A retrospective review described the clinical characteristics of 50 patients, aged 21 years and under, with COVID-19 and who had been admitted to a children's hospital in New York. Among those patients, obesity was the most common comorbidity (22%) along with being overweight (16%). The importance of obesity as an independent risk factor for COVID-19 disease severity has been widely described in adult studies, and it is significant that many of the patients admitted in this study had obesity and/or were overweight. Obesity was also found to be the most significant factor associated with mechanical ventilation in children aged ≥ 2 years [24]. The data suggest that obesity is an important risk factor for the progression of complications due to COVID-19, and in a 2021 review, the authors attempted to understand the possible underlying mechanisms linking obesity to the main complications of the disease by focusing on the metabolic and immune consequences of obesity. The authors reported that obesity increases the risk of SARS-CoV-2 infection and complications through several mechanisms [25]. In adults, firstly, virus entry is promoted by increased levels of ACE2 expression in various cell types, such as pneumocytes and adipocytes. Secondly, it appears that the immune system is unable to provide an adequate immune response, resulting in impaired viral clearance. The authors speculate that the immune system probably overreacts with an overproduction of cytokines by the adipose tissue and immune cells, triggering a cytokine storm [25]. See Figure 8 below.

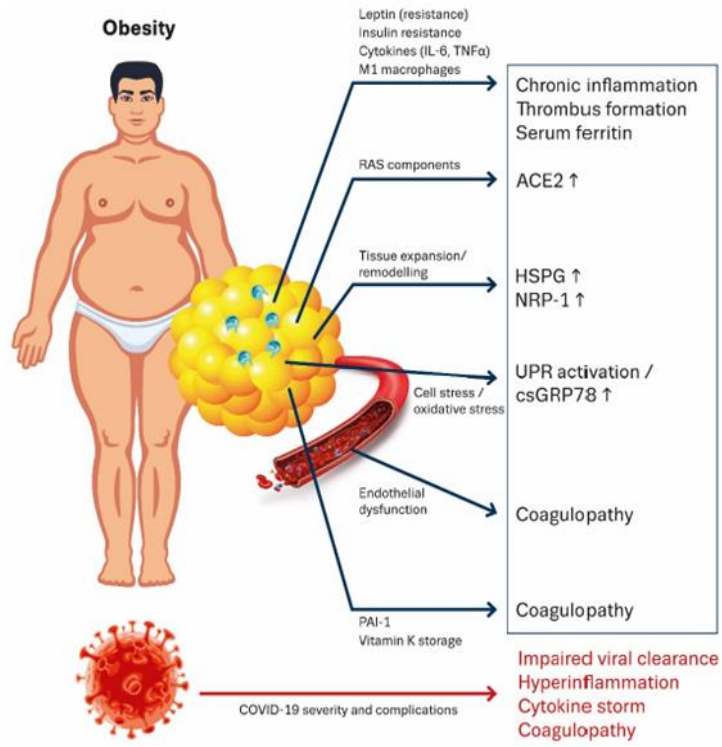

Fig. 8 - The various mechanisms by which obesity may promote COVID-19 disease severity and risk of complications[25]***.*** *Obesity is often accompanied by insulin and leptin resistance, which hinders viral clearance. In addition, obesity is characterised by large hypoxic adipocytes infiltrated by immune cells and M1 macrophages leading to a chronic inflammatory state. The ACE2 produced by adipocytes could provide viral entry into the adipose tissue. In this way, adipose tissue could act as a reservoir for the virus.*

Abbreviations: IL-6 = interleuki-6; TNF = tumour necrosis factor; RAS = renin-angiotensin system; ACE = angiotensin-converting enzyme; HSPG = heparan sulphate proteoglycan; NRP-1 = neuropilin-1; UPR = unfolded protein response; csGRP78 = cell surface glucose-related protein 1 = inhibitor of plasminogen activator 1.

A review published in August 2020, reported that obesity is a highly prevalent comorbidity in severe cases of COVID-19 in children and adolescents. During the COVID-19 outbreak in Canada, obesity was found to be the third most prevalent demographic factor among children admitted to the PICU, after those with severe associated diseases, immunosuppression and cancer [26]. Obesity was the most prevalent comorbidity among the 50 severe cases of COVID-19 affecting American children and adolescents [24].

One of the most relevant aspects for understanding the severity of COVID-19 in obese patients is related to inflammatory issues [27]. The effects of childhood obesity in enhancing severe disease could be explained by the fact that the high visceral adiposity present in obese individuals is known to induce higher levels of local and systemic inflammatory cytokines, such as interleukiω-6 (IL-6) and C-reactive protein (CRP). These cytokines have been positively correlated with the severity of COVID-19, and their higher levels in obese individuals may contribute to their greater susceptibility to severe infections. Obese adult patients are known to exhibit chronic subclinical inflammation, characterised by a permanent low grade inflammatory state [28]. This process is due to cytokines, in particular adipokines with inflammatory properties, produced by adipose tissue and also to a decrease in adiponectin, which has anti-inflammatory properties [29,30] (see Figure 9). SARS-CoV-2 can also lead to beta-cell rupture, through interaction with ACE-2, further aggravating this process [30].

Nutrition plays an important role in the immune and inflammatory response, as certain nutrients modulate cellular and humoral defence systems by modifying the formation of inflammatory mediators, interfering with cellular signal transduction pathways, altering the balance between pro-inflammatory and anti-inflammatory cytokines, and attenuating tissue nutrient depletion [31].

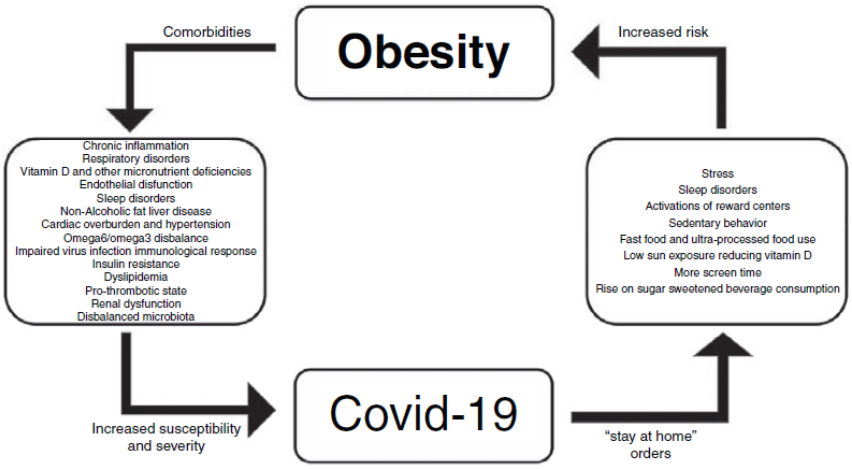

Fig. 9 - Relation between obesity and COVID-19 [31].

1.4 The consequences of the pandemic on physical activity, nutrition and body composition of children and adolescents

Several studies have investigated the indirect effects of the pandemic on children's diet and lifestyle [32]. A survey in Italy conducted during three weeks of home isolation included 41 obese children aged between 6 and 18 years, and showed that the intake of chips, red meat and sugary drinks increased significantly during confinement. This investigation also showed that time spent participating in sporting activities decreased by an average of 2.3 hours/week, and time spent on the screen increased by an average of 4.8 hours/day [33]. Another survey in Italy collected data online on the eating habits and physical activity of 1027 children in Italy aged between 2 and 11 years old during lockdown. This study showed that only 32.3% of the children had a high adherence to the Mediterranean diet, with better scores in children aged 2-5 years, and that 78.1% of the children stopped their usual physical activity, with higher percentages among children aged 6-11 years and children in the north. Only 51.8% maintained some activities in the home, mainly doing exercise/sport. Children spent more time on devices, significantly increasing their sedentariness [34].

A pilot study in Italy of 439 participants aged between 5 and 14 years reported that the basal body weight of 262 (59.7%) children/adolescents (which was 32.2 ± 13 kg) increased. Specifically, 16.2% of the children/adolescents increased their body weight by more than 3 kg, with no significant differences between genders (63% vs. 56%, $p = 0.17$). In addition, the authors found a greater weight gain in adolescents than in children aged < 12 (67% vs. 55%, $p = 0.010$). A subgroup analysis, after excluding obese subjects, found similar results[35].

A review of 84 studies investigated the effects of restrictions on children's physical activity and their determinants. The results showed a decrease in physical activity during the pandemic, ranging from -10.8 min/day to -91 min/day, underlining how time spent on physical activities decreased with increasing age of the children and lower socio-economic background[36].

One of the largest studies comes from the NutriNet-Sante cohort, which surveyed a population of 37,252 French adults and families between March and May 2020. The results suggest that , for a substantial section of the population, lockdown favoured unhealthy nutritional and lifestyle behaviours, such as less physical activity (53%), more sedentariness (63%), more snacking (21%), less consumption of fresh food (27%), more sweets (22%), as well as eating in response to boredom (18%) or anxiety (10%), with an average weight gain of 1.8 kg for 35% of the respondents. The most concerning finding reported by these authors was the persistence of most of these bad habits for more than three months after lockdown: 20% of the children continued to consume unhealthy snacks and 37% of them maintained the habit of spending a lot of time in front of screens [37].

Another study examined the effects of the pandemic on the physical activity and sedentary behaviour of US children, aged 5-13 years, and found that the most common physical activities practised during the initial COVID-19 period were free play/unstructured activities (e.g. running) in 90% of children and walking in 55% of children. Parents of older children (9-13 years) perceived a greater decrease in physical activity and a greater increase in sedentariness from the period before the start of the COVID-19 pandemic than those of younger children (5-8 years) [38]. Within the paediatric population affected by MIS-C , the effects on body composition and nutritional status during hospitalisation and the subsequent months have not been investigated much in the literature. At the same time, nutritional supplementation or interventions have not been reported.

1.5 Nutritional status and risk of malnutrition in hospitalised paediatric patients diagnosed with MIS-C

Adequate nutritional status plays a crucial role in normal growth, response to treatment, comorbidities, quality of life, cost of care, and long-term survival of hospitalised paediatric patients with clinical conditions [39]. In 2013, the Academy of Nutrition and Dietetics and the American Society of Parenteral and Enteral Nutrition (ASPEN) defined paediatric malnutrition as 'an imbalance between nutrient requirements and nutrient intakes, resulting in cumulative deficits of energy, protein or micronutrients that can adversely

affect growth, development and other relevant outcomes'[40], as reported in Figure 10.

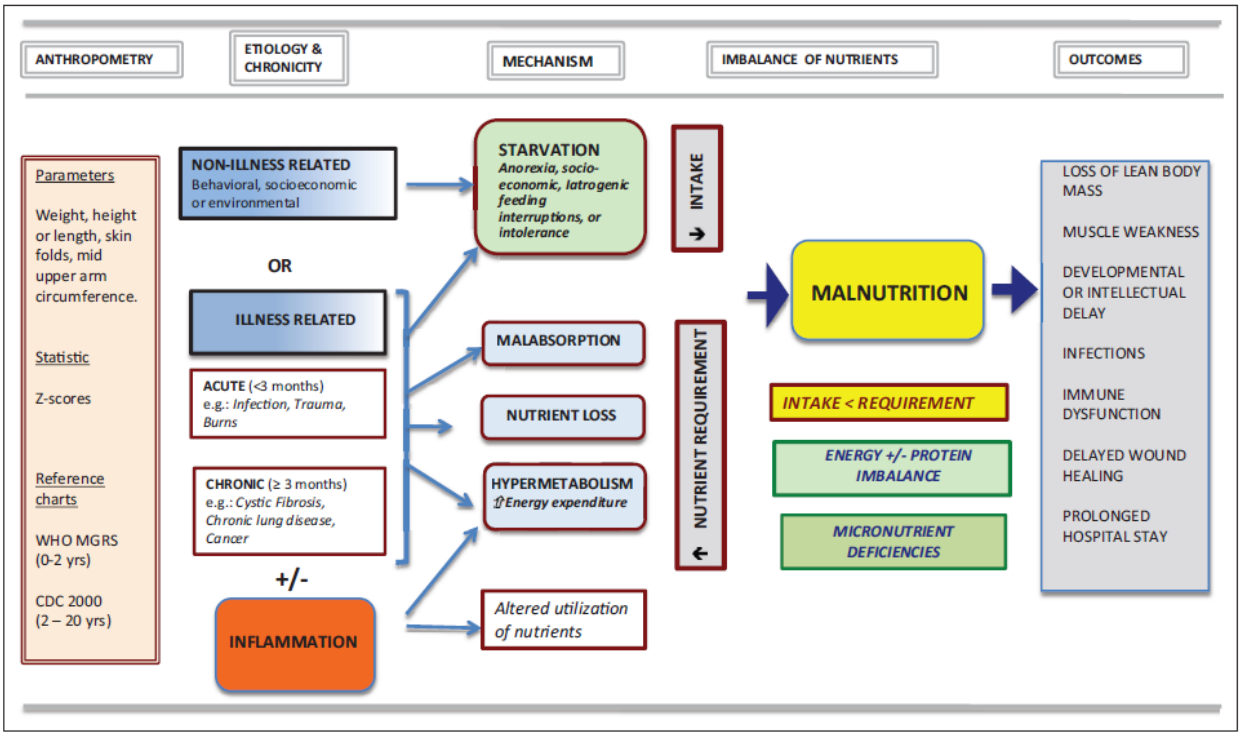

Fig. 10 - Definition of malnutrition in hospitalised children: Key concepts*. CDC, Centers for Disease Control and Prevention; MGRS, Multicenter Growth Reference Study; WHO, World Health Organization*[40].

In paediatric patients, disease-related malnutrition is a dynamic and multifactorial process involving inflammation, nutrient losses, increased energy expenditure, and decreased nutrient intake or utilisation, etc [41]. These conditions can be related to acute diseases (trauma, burns, infections) or chronic diseases (cancer, chronic kidney disease, cystic fibrosis, heart failure, inflammatory bowel disease, neurological and neuromuscular diseases, etc.) [41]. Children have high energy requirements compared to adults and have limited energy reserves. In addition, unlike adults, children need to grow which puts them at a particularly high risk of malnutrition due to their greater needs with an increased likelihood of developing serious nutritional deficiencies when they are hospitalised for longer periods [42].

Malnutrition related to paediatric diseases is still an underestimated problem. This is despite the fact that abnormalities in nutritional status can lead to a significant increase in

co-morbidities and mortality among paediatric patients, and several studies have reported a prevalence of 6%-51% of this condition among hospitalised children [42,43]. Accurate nutritional risk screening and proper assessment of the nutritional status can thus be crucial to the clinical management of these patients [41]. In hospitalised children, early nutritional intervention on body composition is helpful in reversing linear growth arrest, promoting tolerance to therapeutic regimes, improving quality of life, and reducing length of stay [44].

Although anthropometry can provide a general indication of a child's growth status or body size in relation to a reference population, body composition measures of fat mass (FM) and fat free mass (FFM) provide a better understanding of the nutritional status in children with clinical conditions [39]. To prevent malnutrition and, in particular, hospital-acquired malnutrition, the risk of nutritional depletion needs to be identified as early as possible, preferably at the time of admission, so that appropriate nutritional interventions can be initiated promptly [44]. The European Society for Clinical Nutrition and Metabolism (ESPEN) and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommend nutritional risk screening for hospitalised children, to facilitate the identification of children at nutritional risk and to enable the physician to prepare an appropriate nutritional support plan [45].

In 1995, Reilly et al. [46], proposed the Nutritional Risk Score (NRS), based on four main aspects: body mass index (BMI), weight loss in the last three months, food intake in the last week, and severity of illness. This screening is simple to use and applicable to all categories of patients to assess the risk of malnutrition on admission to hospital and to identify the need for nutritional intervention.

The Paediatric Nutrition Risk Score (PNRS), proposed in 2000 by Sermet-Geydelus et al. [47], analyses three items: the patient's medical condition (rated from 1 to 3 points according to the presence of mild, moderate or severe disease), the presence of pain (1 point if pain is present), the reduction in food intake (1 point if food intake is <50%). A score ≥3 indicates that the patient is at high risk of malnutrition and should be referred to a nutritionist team.

This method is quick to perform but does not identify the nutritional status of the patient. McCarthy et al. [48], validated the Screening Tool for the Assessment of Malnutrition Paediatrics (STAMP) in a UK study of medical and surgical patients aged between 2 and 17 years. The score assesses the patient's clinical diagnosis, nutritional intake during hospitalisation and anthropometric measures, developing a care plan based on the child's overall risk of malnutrition (low, medium or high). Gerasimides et al. [49], adopted the Paediatric Yorkhill Malnutrition Score (PYMS) in the UK among patients aged 1-16 years. The PYMS assesses four items: BMI, history of recent weight loss, changes in nutritional intake, and the expected effect of the current medical condition on the patient's nutritional status. The total score reflects the patient's degree of nutritional risk.

In a multicentre study conducted in the Netherlands on medical and surgical patients aged between 1 month and 18 years, Hulst et al. [50] proposed the Screening Tool for Impaired Nutritional Status and Growth (STRONGkids). This tool consists of four items: subjective clinical assessment; high-risk diseases; nutritional intake and losses; and weight loss or low weight gain. This tool was found to be quick and easy to use (taking an average of three minutes), and can also predict the length of hospitalisation and identify the need for nutritional interventions [50].

In hospitalised paediatric patients, a comprehensive nutritional assessment is also necessary, including medical and dietary history, a detailed physical examination with accurate anthropometric measurements (weight, height, head circumference, body mass index, arm circumference and triceps skinfold), medical examination results and body composition assessment, as no single parameter is a complete indicator of nutritional status. In addition, body composition analysis requires the assessment of fat mass (FM), lean body mass (FFM), and body cell mass (BCM) [41]. According to ESPEN statements, nutritional risk screening tools are designed to detect protein and energy malnutrition and/or predict whether malnutrition may develop or worsen [41].

Huizar et al., highlight that food insecurity, malnutrition, and obesity, independently represent three of the most complex conditions that threaten the livelihood of populations

in almost every country, making them the leading causes of ill health globally. The most detrimental impact of the complex phenomenon of food insecurity on individual health is the increased likelihood of malnutrition, which is most often associated with undernutrition (i.e., wasting, stunting, underweight). However, malnutrition has been increasingly recognised as a physiological consequence of overnutrition, with evidence suggesting its role in both the development and progression of diet-related diseases (e.g., obesity, cardiovascular disease, diabetes, some cancers) and premature mortality. Malnutrition by excess or undernutrition can cause fat accumulation and loss of lean mass, thus affecting the quality of body composition [51].

The body composition of an individual exposed to the virus plays an important role in the response to infection. Fat accumulation and loss of lean body mass can affect the functioning of the entire body, both in the short term, by influencing susceptibility and immunological response to the virus, inflammatory reaction, and metabolic and respiratory distress, and in the long term by conditioning disease outcomes, such as the time required for full recovery, or the risk of long-term disability [51].

In adults, malnutrition has been associated with a higher risk of contracting SARS-CoV-2 infection and, also, more severe cases (e.g. hospitalisation, mechanical ventilation) [52] . It has been suggested that malnutrition contributes to obesity, just as obesity contributes to malnutrition, and both are driven by unhealthy eating behaviour, especially in the presence of food insecurity. These different conditions can increase the risk of negative health outcomes [53].

In adults, ectopic fat deposition can lead to organ failure in a pro-inflammatory environment, with an increase in adipokines and inflammatory cytokines such as TNF-alpha, leptin and IL-6, resulting in impaired immune response to SARS-CoV-2 infection and severe complications [51]. In contrast, in a study of two independent cohorts of children and adolescents aged 1-18 years admitted to intensive care units (ICU) for neurological, respiratory and cardiovascular diseases, a high BMI at baseline was found to be significantly associated with persistent functional impairment acquired at hospital

discharge. Through body composition analyses, performed by computed tomography (CT) scanning the area of skeletal muscle and fat, the authors demonstrated that two different anthropometric phenotypes - high skeletal muscle mass and high visceral fat mass - were independently associated with persistent functional impairment [54].

Kurtz et. al showed an association between previous diagnoses of malnutrition and the development of severe COVID-19 with patient age in both paediatric and adult settings [55]. Their study highlighted that the odds of severe COVID-19 increased among malnourished adults (adults with a history of malnutrition) between the ages of 18 and 78 years and at higher values than among their peers without a history of malnutrition. A crossover effect occurred at age 79, when patients without a history of malnutrition had higher odds of severe COVID-19 than their malnourished peers, although the risk in both groups continued to increase with age.

In the paediatric population, on the other hand, the effect of a malnutrition diagnosis on severe COVID-19 is greater in younger children (less than 5 years) [55]. Children who are not at risk of malnutrition (or who do not have a history of malnutrition diagnosis) have a lower risk of developing severe disease with increasing age, and patients older

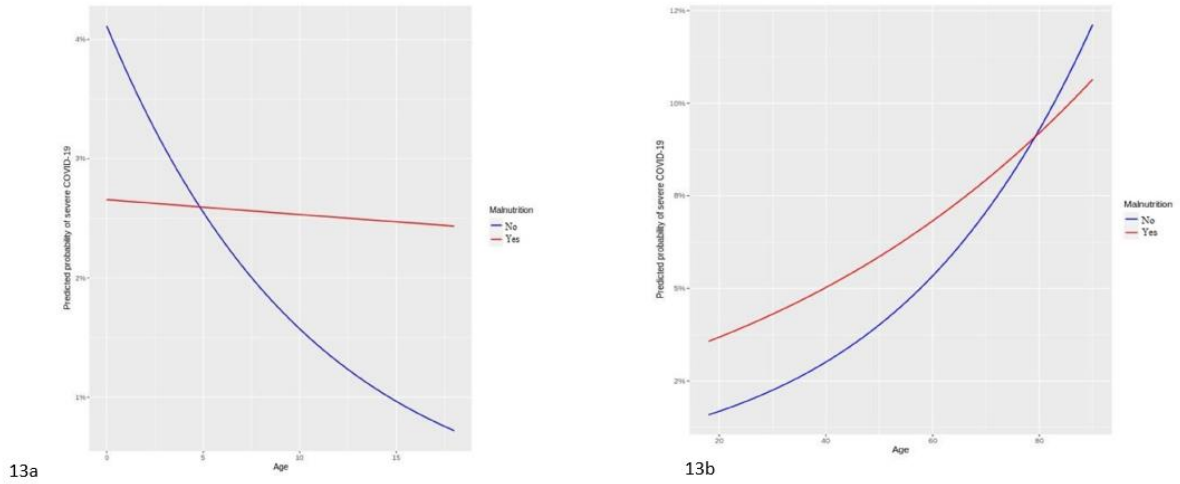

Fig. 11 - *Interaction between COVID-19 disease severity and malnutrition in children (13a) and adults (13b)* [55].

than five years have a lower risk of developing severe disease than their peers with malnutrition [55]. Consequently, all children at risk of malnutrition require care and

nutritional support to meet their nutritional needs [55]. Figure 11 shows the interaction between COVID-19 disease severity and malnutrition in children (13a) and adults (13b).

1.6 Glucose impairment during inflammatory status in MIS-C children

The literature suggests that COVID-19 and metabolic glucose impairment actively interact [56]. In addition to the significant systemic inflammation reported in COVID-19 patients as a source of stress-induced hyperglycaemia, a recent study found that SARS-CoV-2 can directly infect pancreatic β-cells, leading to β-cell malfunction and insulin insufficiency [57,58]. This suggests that there may be a bidirectional relationship between hyperglycaemia and COVID-19 severity. Children were less impacted than adults, and SARS-CoV-2 was typically asymptomatic or had minor symptoms [59]. However, SARS-CoV-2 infection appears to cause children to develop a severe multisystem inflammatory syndrome (MIS-C) [60]. Due to peripheral insulin resistance (IR), relative insulin deficiency, and impairment in glucose metabolism, hyperglycaemia is a common complication in critically ill non-diabetic children [61,62]. The effects of medications such as catecholamine, glucocorticoids, and exogenous dextrose administration should also be taken into account. Blood glucose fluctuation is linked to multiorgan dysfunction, and prolonged duration of hyperglycaemia is an independent factor related to mortality[63]. This is despite the fact that strict glucose management is not significantly correlated with a reduction in hospital mortality[64]. Although MIS-C is a serious health disease linked to SARS-CoV-2 infection, information on glucose abnormalities is still lacking.

1.7 Polyunsaturated fatty acids (PUFAs) in inflammatory status and Sars-Cov 2

Inflammation or phlogosis is a core mechanism of innate immunity, which occurs in the presence of pathogens and tissue damage [65]. Inflammation enables the underlying trigger to be removed, tissue damage to be repaired, and normal body function to be re-established thanks to the intervention of defensive cells. It is characterised by flushing, heat, pain, swelling, and loss of tissue function [65] . The inflammatory response consists of increased

blood flow and increased permeability of blood capillaries, enabling large molecules to leave the blood stream and cross the endothelial wall. The response also enables and the movement of fluids, proteins, and leucocytes from the blood stream to the site of tissue damage [65].

An inflammatory response that lasts only a few days is called acute inflammation, while a response of longer duration is called chronic inflammation [66]. Acute inflammation causes symptoms, usually temporary, that disappear when the inflammatory response is complete. In some cases, however, acute inflammation can cause damage, such as tissue destruction or a prolonged and damaging inflammatory response. This occurs when the regulatory mechanisms of the inflammatory response are defective or the ability to eliminate the harmful agent is impaired [66].

The inflammatory process has several stages that occur in a specific time sequence. First there is a vascular response by vasodilatation, which leads to an increase in blood flow, followed by increased permeability of the microcirculation and the formation of exudate, which lead to the leakage of the liquid part of the blood and an increase in extravascular fluid that is rich in plasma proteins, respectively. Subsequently, the phenomenon known as diapedesis, i.e. leucocyte extravasation, occurs [65]. In order to ensure that the inflammatory process continues, and the elements of damage can be removed, diapedesis must be followed by chemotaxis, which enables the accumulation of leucocytes at the site of damage, following the release of chemical mediators. At this point, the process involves phagocytosis, i.e. the ingestion and destruction of pathogenic microorganisms and cellular debris. This is followed by the resolution or chronicity of the inflammatory process [65].

Polyunsaturated fatty acids (PUFAs) play a key role in the inflammatory process and its resolution [67]. The link between PUFAs and inflammation are the eicosanoids, mediators and regulators of inflammation (see Figure 12). Since inflammatory cells typically contain a high proportion of arachidonic acid (AA 20:4-n6) and low proportions of other PUFAs, AA is usually the main substrate for the synthesis of eicosanoids [68]. Eicosanoids, which include prostaglandins (PGs), thromboxanes, leukotrienes (LTs) and other oxidised

derivatives, are generated from AA by metabolic processes. AA is released by the action of phospholipase A2 (PLA2) from membrane phospholipids. AA can be converted by cyclooxygenase (COX) to various PGs, or by 5-lipoxygenase (5-LOX) to LT 43. Eicosanoids can also originate from eicosapentaenoic acid (EPA 20:5ω-3). However, due to structural differences between EPA and AA, they generate eicosanoids with different structures [69]. AA gives rise to PGD2 and LTE4; and EPA gives rise to PGD3 and LTE5. Eicosanoids are involved in modulating the intensity and duration of inflammatory responses and they often have opposite effects .The eicosanoids produced by EPA have less biologically impact than those produced by AA, on the contrary resolvins and protectins produced by DHA have a great anti-inflammatory action. ,

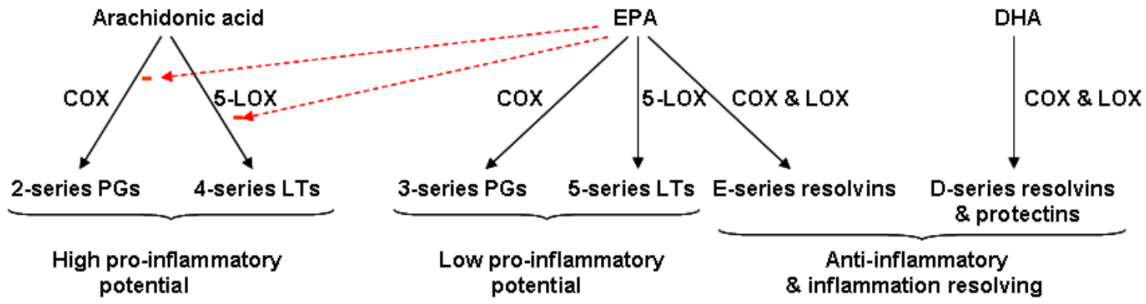

Fig. 12. *General overview from Calder et al. [67] of synthesis and actions of lipid mediators produced by AA, EPA and DHA. COX, cyclooxygenase; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin.*

The overall physiological (or pathophysiological) outcome thus depends on the cells present, the nature of the stimulus, the timing of eicosanoid generation, the concentrations of the different eicosanoids generated, and the sensitivity of the target cells and tissues to them. For example, PGE2 i) induces cyclooxygenase 2 (COX-2) in fibroblast cells (by increasing its production); ii) induces IL-6 production by macrophages; iii) inhibits 5-lipoxygenase (5-LOX) and thereby decreases the production of leukotrienes (LT) series 4; iv) induces 15-LOX; and v) promotes the formation of lipoxins that exhibit anti-inflammatory effects [68]. PGE2 thus possesses both pro- and anti-inflammatory actions.

Excessive production of AA-derived eicosanoids (PG and LT) is associated with inflammatory diseases. For instance, patients with inflammatory bowel disease (IBD) show

high levels of PG and other AA-derived eicosanoids in the inflamed mucosa, which correlates with disease activity. Moreover, in the presence of an injurious agent, chemotactic factors are produced that recruit leukocytes to the inflammation and trigger the initiation of an adaptive response [67]. The migration of leucocytes from the bloodstream to the inflammatory tissue is a key process involving adhesion molecules. ω-3 PUFAs can reduce the expression of adhesion molecules in the endothelium, antagonise the metabolism of ω-6 PUFAs, and lead to the generation of eicosanoids with fewer inflammatory actions [70]. For example, the migration of neutrophils across the endothelium barrier requires the binding of PGD2, an AA-derived eicosanoid, to the leukocyte DP1 receptor.

EPA inhibits the pathway that leads to the production of PGD2 from AA, and leads to the generation of PGD3, which antagonises the PGD2 receptor [71]. Although the role of PGs in initiating the inflammatory phase is well known, PGs are also involved in the resolution of inflammatory processes and are a critical component in wound healing, tissue regeneration and fibrosis. For instance, PGs also initiate the repair and protection of gastrointestinal mucosa. In fact, PG production by mesenchymal stem cells leads to the release of growth factors by myofibroblasts. Similarly, PGE2 has been shown to switch from the stimulator to inhibitor of cell migration after the epithelial-mesenchymal transition of airway epithelial cells, a key process during wound healing. There is a notable interaction between the various AA-derived eicosanoids [71]. LTE4 is produced by mast cells and immune cells and has a strong bronchoconstrictor effect. LTE4 can regulate COX-2 expression, and subsequently generate PGD2 via a PPARγ-dependent mechanism in mast cells [71].

Omega-3 PUFAs (ω-3 PUFAs) are thought to be anti-inflammatory. The anti-inflammatory effects of ω-3 PUFAs may be mediated by competition with Omega-6 PUFAs (ω-6 PUFAs), as ω-3 PUFAs act as a competitive substrate for the metabolism of ω-6 PUFAs (see Figure 13).

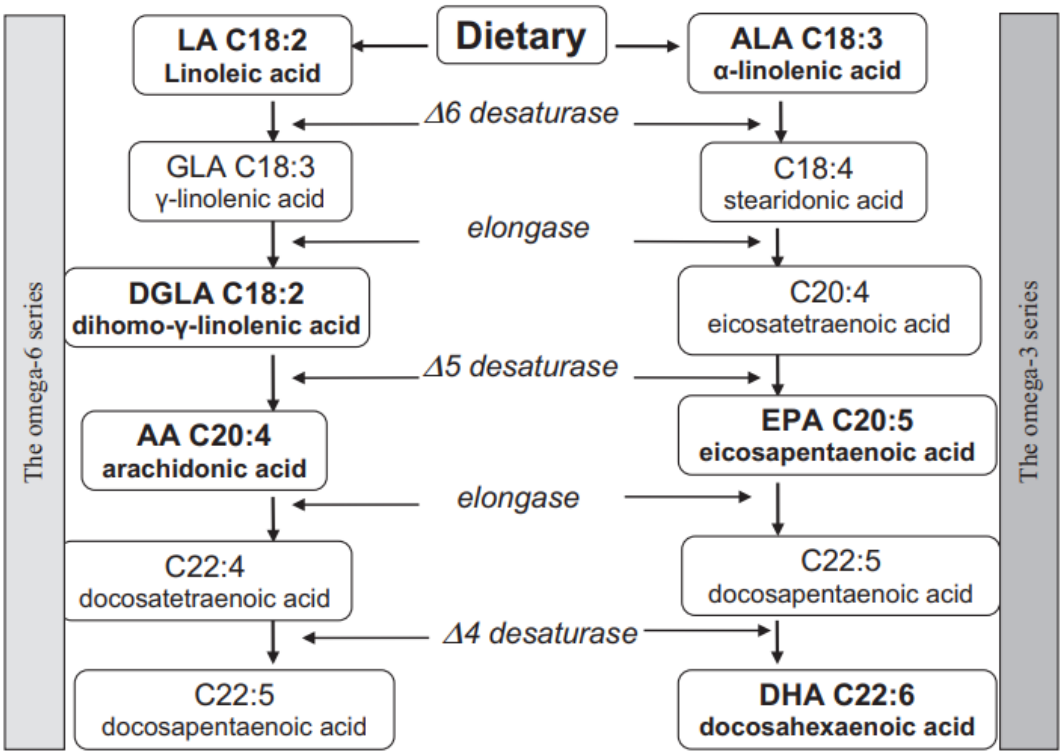

Fig. 13 *Pathways of biosynthesis of the PUFA omega-6 and -3 series*[72].

Experimental evidence suggests that nutritional intervention with ω-3 PUFAs reduces AA-derived eicosanoids, while increasing the cellular content of ω-3 PUFAs [73] . Increasing the content of EPA and docosahexaenoic acid (DHA 22:6 ω-3) in the membranes of cells involved in inflammation, affects the nature of membranes and the formation of signalling platforms called lipid rafts. EPA and DHA interfere with the metabolism of AA, which produces prostaglandins and leukotrienes involved in inflammation. EPA gives rise to weak (less inflammatory) analogues, and both EPA and DHA are substrates for the synthesis of specialised pro-resolution mediators (SPMs). SPMs include the E- and D-series resolvins, produced by EPA and DHA respectively, and the protectins (also known as neuroprotectins) and maresins produced by DHA.

Resolvins and protectins are very powerful anti-inflammatory compounds. Protectins are also involved in wound healing. In addition to their anti-inflammatory properties, resolvins can reduce insulin resistance by up-regulating adiponectin or by increasing leptin levels in adipocytes[74]. The generation of eicosanoids involves the COX or LOX pathways, which operate separately from each other. However, SPMs are synthesised using COX and

LOX enzymes in the same pathway. The net result is a reduction in the production of inflammatory cytokines, chemokines, adhesion molecules, proteases, and enzymes.

The anti-inflammatory and resolving effects of EPA and DHA are important for both the prevention and treatment of human diseases that have an inflammatory component. The anti-inflammatory properties of ω-3 FAs, particularly EPA, are due to competition with AA as a substrate for cyclooxygenases and 5-lipoxygenases. Eicosanoids derived from ω-6 and ω-3 FAs have opposing properties and are recognised to be the meeting point between PUFAs, inflammation and immunity. In addition to their effects on prostaglandins, thromboxanes and leukotrienes, ω-3 FAs suppress the production of interleukin 1 (IL-1), thereby suppressing IL-1-induced Cox2 (cyclooxygenase) expression. α-linolenic acid (ALA 18:3n-3), EPA and DHA are also involved in the immune function [75].

The precise efficacy of ALA depends on the level of linoleic acid (LA) and the total PUFA content in the diet. It is unclear whether ALA itself exerts these effects or whether they are the result of its conversion to EPA [76] . The excessive intake of ω-6 PUFAs, which is characteristic of Western diets, produces an imbalance between ω-6 and ω-3 PUFAs that leads to an overproduction of pro-inflammatory ω-6 series prostaglandins and cytokines. LA-rich vegetable oil supplements increase IL-1 and TNF [77]. Subjects given ω-3-rich flaxseed oil or fish oil supplements drastically reduced IL-1, IL-2 and TNF production, as well as suppressing mononuclear cell proliferation and IL-2 receptor expression.

This thus suggests that LA increases the secretion of proinflammatory cytokines, while fish oil reduces the secretion of proinflammatory cytokines. The introduction of ω-3 PUFAs appears to modify inflammatory and immune reactions, making ω-3 PUFAs potential therapeutic agents for inflammatory and autoimmune diseases [78,79]. Their effects are determined by modulating the type and amount of eicosanoids and cytokines, and by altering gene expression [80]. For instance, maresin-1, a derivative of DHA, has shown beneficial actions in respiratory diseases, arthritis and cardiovascular diseases from preclinical and animal studies [81–84].

In adult patients suffering from cardiovascular diseases, cancer, obesity, arthritis, inflammatory bowel disease, psoriasis, asthma, rheumatological diseases, sarcopenia, cognitive decline and depression, several studies have been conducted on ω-3 supplementation [85]. When given in the right amounts, EPA and/or DHA may enhance outcomes in critically sick patients with ARDS, sepsis, and organ injury, according to a recent overview by Singer P. et al. [86] .

Nutritional supplementation with ω-3 PUFAs as an alternative or adjunctive therapy is gaining interest in clinical practice, especially since current drug therapies have many side effects and diseases are heterogeneous. In children, ω-3 supplementation is employed in clinical practice in various pathologies such as cystic fibrosis [87] , cancer [88] , or inborn metabolic diseases [89] . With regard to inflammation-based diseases, EPA and DHA have been used to treat obesity, hypercholesterolaemia, and NAFLD [90–93] . In healthy children, widely varying levels of fatty acids have been found, as shown in Table 2 below.

Table 2 : Fatty acids values in children founded in the literature.

Group-Author	**Risé [94]**	**Crippa[95]**	**Bonafini [96]**	**Van der Wurff [97]**	**Ryan[98]**
Age (year)	*<9*	*7-14*	*7-9*	*13-15*	*4*
% LA		22.54±2.45			
<9 y	17.6±1.92		19.9±2.32		
% AA		10.10±0.92			
<9 y	8.33±1.04		12.21±1.67		7.50±1.89
> 9 y				11.01±11.33	
% ALA					
<9 y	0.15±0.05		0.16±0.08		
% EPA		1.13±0.45			
<9 y	0.23±0.08		0.30±0.17		0.30±0.39
>9 y				0.34±0.42	
% DHA		1.93±0.53			
<9 y	1.40±0.37		2.92±0.76		1.00±0.34
>9 y				2.49±2.63	

1.7.1 PUFAs and Sars-Cov-2

During the COVID-19 pandemic, ω-3 plasma levels in the adult population and the risk of COVID-19 outcomes (testing positive for SARS-CoV-2, hospitalization, and death) were assessed by various research groups. Harris et al. evaluated the FA levels in adults from the UK biobank. A strong inverse and dose-related relationship was found between plasma DHA% and the risk of being positive to Sars-Cov-2 and hospitalization or death due to Covid-19. The authors stated that dietary interventions to increase the circulation of ω-3 PUFA levels, such as increased fatty fish consumption and/or ω-3 FA supplementation, might reduce the probability of unfavourable COVID-19 outcomes.

Observational studies comparing COVID-19 patients to healthy controls and across severity of patient subgroups, have observed significant variations in the circulation levels of several PUFAs [99,100] . On average, COVID-19 patients had lower levels of total PUFAs, ω-6 PUFAs, linoleic acid (LA), and the omega-3 index (O3I), calculated as the percentage of EPA and DHA in red blood cell (RBC) fatty acids [101] . A lower probability of the need for mechanical breathing and of death was also linked to a higher O3I in individuals [102,103]. A pilot study on 100 adults suggested that RBC O3I was inversely associated with the risk of death. A higher odds ratio for death was found in patients with the lowest O3I [104].

The biological mechanism has been investigated by Huang et al. , who found that ω-3 PUFA decreased ACE2 protein levels and prevented Sars-Cov-2 entry in human endothelial, kidney cell lines, and human plasma [105] . In addition, EPA and DHA inhibited the conversion of SREBP1 from its inactive form to the mature active form and increased the degradation of SREBP1-1c mRNA [106] (Figure 14) .

It is important to note that the ω-3 PUFA dietary intake levels of countries in the West remain far below current recommendations, taking into account both the ω-3 precursor ALA and long chain derivatives such as EPA and DHA [106]. As a result of significant changes in agricultural production and consumption habits over the last 50 years, today's consumption of ω-6 PUFAs is at least equal to, and frequently exceeds, the current recommendation (depending on the country), whereas consumption of ω-3 PUFAs is

almost always deficient in the West [107]. This has resulted in a high (10 or more) ω-6/ω-3 ratio since the 1980s[108].

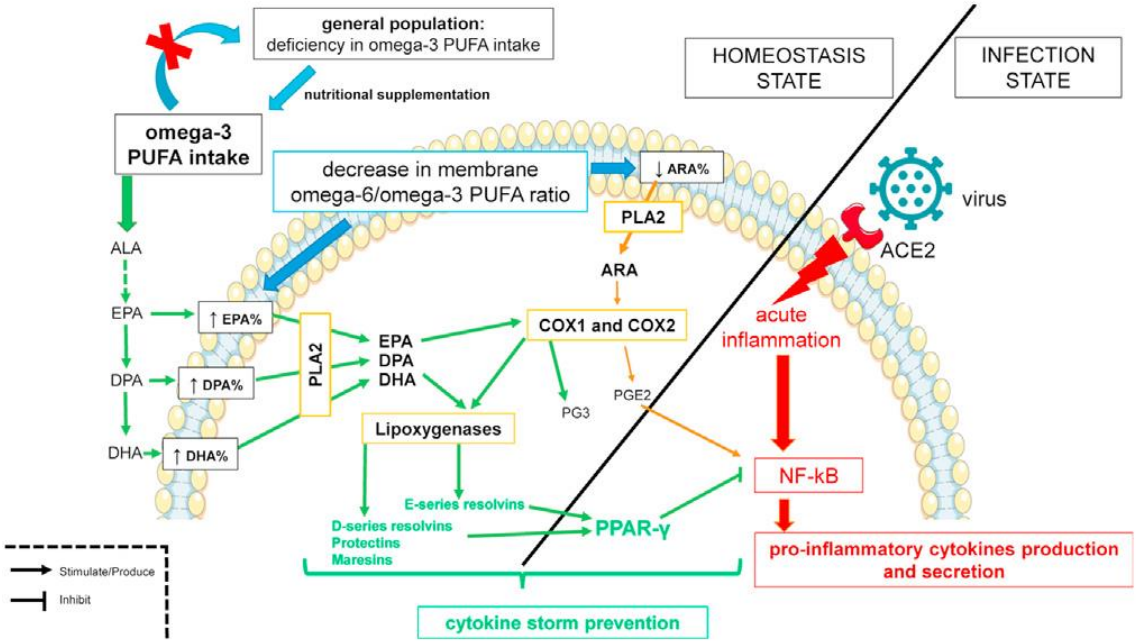

Fig. 14. *Mechanism of the anti-inflammatory effects of ω-3 LC-PUFAs and cytokine storm prevention in the Covid-19 virus*[106].

Sertoglu et al. analyzed the IL-6 of 106 patients with Covid-19 , and their fatty acid profile. Their results showed a moderately negative correlation between total ω-3 and IL-6 and procalcitonin, together with positive correlations with the ω-6/ω-3 ratio inflammatory markers [109]. They concluded that the ratio of arachidonic acid to eicosapentaenoic acid, and ω-3 PUFAs, can be a systemic sign of poor prognosis, increased lung damage, and high mortality in COVID-19, together with IL-6 [109]. In critically ill adult patients with Covid-19, the effect of ω-3 supplementation during hospitalisation has been tested in relation to both the severity of the disease and the inflammatory parameters. A Cochrane meta-analysis of 10 studies found a statistically significant decrease in ARDS mortality when ω-3 PUFAs were compared to a lipid-rich enteral formula. However this meta-analysis also noted that the studies were highly heterogeneous, making it difficult to determine whether ω-3 PUFA supplementation affects mortality, oxygenation, or the length of mechanical ventilation

and ICU stays [110]. The COVID-Omega-F Trial tested whether ω-3 PUFA emulsion containing 10 g of fish oil per 100 mL, of which 1.25–2.82 g DHA and 1.44–3.09 g EPA (0.2 g/kg/day at 0.5 mL/kg/h) had an effect on inflammatory biomarkers, pro-resolving mediators and FA levels, compared to the placebo (i.v. NaCl at 0.5 mL/kg/h to equivalent volume). However, there are still no published results available on the effectiveness of this treatment [111].

1.8 State of the art

In the paediatric population, it is common for the acute disease event not to be associated with the possible risk of malnutrition or sarcopenia. It is usually rare for healthy children who are affected by an acute event to experience sarcopenia or underestimated body weight loss [112]. Instead, sarcopenia typically occur in those sections of the population already suffering from other chronic diseases, transplantation or limited motor skills [113]. Moreover, during the pandemic, the elderly population was the most affected and for whom the risk of sarcopenia was immediately identified due to prolonged hospitalisation and admission to an intensive care unit[114].

With the emergence of this new paediatric multi-inflammatory disease, MIS-C, paediatric patients also had to be hospitalised. In the literature, however, the risk of malnutrition and sarcopenia, as well as the role of certain nutrients, did not seem to be considered for children, mainly for adults.

Our aim is to verify the utility of assessing the risk of malnutrition and then to identify an early intervention for better recovery. In addition, in children suffering from MIS-C, it has been assumed that the FA levels are severely altered during the acute phase and that the severe inflammation may subsequently require ω-3 FA supplementation. However, the effects of ω-3 FA supplementation in acute inflammation and during follow-up have not been investigated, probably due to the reduced prevalence of the condition and the difficulty in conducting a trial in the paediatric population.

Chapter 2

Aim of the study

The aim of this study is to evaluate, during hospital stay and longitudinally (up to 6 months after hospital discharge), the risk of malnutrition, the body composition, and the FA profile in a cohort of children with MIS-C diagnosis, admitted at Vittore Buzzi Children's Hospital from December 2020 to February 2022. In addition, we wanted to investigate the effectiveness of DHA on inflammatory markers after 3 months of supplementation, in a subsample of our cohort.

Chapter 3

Materials and methods

3.1 Study design

This study was conducted on a total of 58 patients, children and adolescents aged between 1 and 17 years, who were admitted to the Paediatrics Department of the Vittore Buzzi Hospital in Milan with a diagnosis of MIS-C, in accordance with the criteria developed by the CDC (CDC, 2020). For all patients, a clinical and biochemical assessment was recorded on admission. Moreover, anthropometric measurements were collected at time of admission and before hospital discharge. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the hospital (protocol number 2021/ST/004). Children's caregivers gave their written consent for inclusion after being informed about the nature of the study.

3.2 Anthropometric data collection and malnutrition risk assessment

Anthropometric measurements were performed for all patients on admission (T0), at discharge (T1), 10 days (T2), 1 month (T3), 3 months (T4) and 6 months (T5) after discharge. For the assessment of body composition, anthropometric parameters were measured. Weight and height were measured using a mechanical column scale with altimeter (Seca 711 and Seca 220); the body mass index (BMI , kg/m2) and the BMI z-score (BMIz) were established according to the WHO growth reference values (WHO Multicentre Growth Reference Study Group, 2006); circumferences of the arm (CB), waist (CV), wrist and hips were measured with a tape measure (Seca 201); skinfolds (tricipital, bicipital, subscapular and suprailiac) were measured with a plicometer (Holtain 610). The z-scores for the tricipital plica (PTz) were calculated [114] and for the arm circumference (CBz) [115]; The measured skinfolds were subsequently used to estimate body composition using the predictive equations for children and adolescents from which fat mass (FM) and lean mass (FFM) can be obtained, using the specific equations for the paediatric population. Fat mass index (FMI) and lean mass index (FFMI) were also calculated [116–119] .

For the assessment of nutritional risk, the StrongKids *Screening Tool for Risk On Nutritional status and Growth)* questionnaire was carried out on admission and repeated before discharge [49] . **See Figure 15.**

Screening risk of malnutrition Asses following items < 24h after admission and once a week thereafter	Score →points	
1. Is there an underlying illness with risk for malnutrition (*see list*) or expected major surgery?	No	Yes → 2
2. Is the patient in a poor nutritional status judged with subjective clinical assessment: loss of subcutaneous fat and/or loss of muscle mass and/or hollow face?	No	Yes → 1
3. Is one of the following items present? ▪ Excessive diarrhoea (≥5 per day) and/ or vomiting (> 3 times/ day) during the last 1-3 days ▪ Reduced food intake during the last 1-3 days ▪ Pre-existing nutritional intervention (e.g. ONS or tube feeding) ▪ Inability to consume adequate nutritional intake because of pain	No	Yes → 1
4. Is there weight loss (all ages) and/or no increase in weight/height (infants < 1year) during the last few week-months?	No	Yes → 1

Maximum total score: 5 points

Risk of malnutrition and need for intervention		
Score	**Risk**	**Intervention and follow-up**
4-5 points	**High risk**	• Consult doctor and dietician for full diagnosis and individual nutritional advice and follow-up. • Check weight twice a week and evaluate nutritional advice • Evaluate the nutritional risk weekly
1-3 points	**Medium risk**	• Consider nutritional intervention • Check weight twice a week • Evaluate the nutritional risk weekly
0 points	**Low risk**	• No nutritional intervention necessary • Check weight regularly (according to hospital policy) • Evaluate the nutritional risk weekly

Fig. 15 - StrongKids questionnaire used for malnutrition risk assessment [49]

3.3 Blood drops collection for fatty acids assessment

After 5–7 days from admission (T0), a drop of blood was collected on Guthrie Test paper from each patient and stored in a refrigerator until analysis as described below. At this time, the drug therapy of children was also recorded. The Guthrie Test was collected again at the 3-month(T1) and 6-month(T2) follow-ups. The FA profile was evaluated in a drop

of blood collected on a Guthrie paper embedded with butylated hydroxy toluene (BHT) as antioxidant. After direct transmethylation, FA methyl esters were analyzed by gas chromatography using a GC-2100 (Shimadzu Italia S.r.l., Milano, Italy) equipped with a 15 m capillary column (DBB Agilent), PTV injector and FID detection [120,121]. Relative percentages were used to report 23 FA; total saturated FA (SAT), monounsaturated FA (MUFA) and PUFA were also reported. In addition, the O3I was calculated in accordance with Stark et al. [122].

3.4 Statistical analysis

Anthropometric data: statistical analysis was performed using STATA version 12.0 (StataCorp, College Station, TX, USA). Continuous variables were checked for normality using the Kolmogorov-Smirnov test. As no variations from normality were observed, all continuous variables are presented as mean and standard deviation. Coarsened exact matching (CEM) was used to match patients with MIS-C with control subjects on sex, age, and BMI. A t-test for independent samples was used to test differences in outcomes of interest between patients with MIS-C and control patients. Pearson or Spearman (for dichotomous variables) correlation was used to test the association between anthropometry and body composition at hospitalization and STRONGkids with disease outcomes (length of hospitalization, day of ventilation, PICU stay). A paired t-test was used to test changes in outcomes of interest between hospitalization and hospital discharge in patients with MIS-C. To test BMI changes during hospitalisation and changes over time of the outcomes of interest from hospital discharge a repeated-measures ANOVA, followed by a Bonferroni's multicomparison test, was used. To test the effect of potential confounders (duration of hospital stay, pre-admission BMI, gender, age, nutrients intake, PICU stay and time on ventilation) on anthropometrical measurements changes, mixed-effects linear regression models were used.

Fatty acids analysis: a descriptive statistical analysis was performed using IBM SPSS statistics version 27, whereas Spearman's correlation coefficients were estimated for biochemical parameters and FA.

Chapter 4

Anthropometric measurements and body composition in children with MIS-C

4.1 Introduction:

As the prevalence of MIS-C is low, there are no data in the literature on the effects of MIS-C on body composition and the risk of malnutrition. Using state surveillance data for MIS-C in' of the USA, the incidence was estimated to be about 3 cases per 10,000 individuals under the age of 21. Limited data suggest an incidence rate of 0.045% (95% confidence interval, 0.035%-0.068%) in England during the first wave, or 4.5 cases per 10,000 SARS-CoV-2 positive individuals. All estimates to date have led to the conclusion that MIS-C is a relatively rare complication of SARS-CoV-2 infection in children [115]. However, in the acute phase of the disease, children experience a severely inflammatory condition, with a long period of hospitalization. A long hospital stay is associated with a decrease in weight due to the loss of lean mass caused by restricted movement and the ongoing pathology. In addition, the food intake in the acute phase of the pathology may be very limited.

Even after hospital discharge there may only be a partial recovery of muscle mass or sarcopenia-related symptoms, due in part to the limitation of spontaneous and structured physical activity suggested by the European Society of Cardiology [116].

For this reason, the risk of malnutrition and body composition over time was assessed in order to observe whether the healthy children with a diagnosis of MIS-C might have been affected by malnutrition and body composition changes over time, and what the subsequent implications might have been on the children's body composition because of the pandemic restrictions.

4.2 Manuscript 1

"Longitudinal anthropometry and body composition in children with SARS-CoV-2-associated multisystem inflammatory syndrome"

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Longitudinal Anthropometry and Body Composition in Children With SARS-CoV-2-Associated Multisystem Inflammatory Syndrome

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ABSTRACT

Objectives: Acute coronavirus disease 2019 infection has been shown to negatively affect body composition among adult and malnourished or obesity children. Our aim is to longitudinally evaluate body composition in children affected by the Multisystem Inflammatory Syndrome (MIS-C).

Methods: In this cohort study, we recruited 40 patients affected by MIS-C, aged 2–18 years old, who were admitted in our clinic between December 2020 and February 2021. Physical examination for each participant included weight, height, body mass index (BMI) *z* score, circumferences, and skinfolds assessment. The same measurements were repeated during outpatient follow-up at 10 (T2), 30 (T3), 90 (T4), and 180 (T5) days after hospital discharge. Fat mass and fat free mass were calculated according to skinfolds predictive equations for children and adolescents. A control group was randomly selected among patients attending a pediatric nutritional outpatient clinic.

Results: BMI *z* score significantly decrease between preadmission and hospital discharge. Similarly, arm circumference *z* score, arm muscular area *z* score, and arm fat area *z* score significantly decreased, during hospital stay. Fat mass index (FMI) significantly increased over time, peaking at T3. Fat free mass index decreased during hospitalization.

Conclusions: To the best of our knowledge, this is the first study to assess body composition in a numerically large pediatric MIS-C population from acute infection to 6 months after triggering event. FMI and anthropometric parameters linked to fat deposits were significantly higher 6 months after acute event. Thus, limiting physical activity and having sedentary lifestyle may lead to an accumulation of adipose tissue even in healthy children who experienced MIS-C and long hospitalization.

What Is Known

- Malnutrition can negatively impact the severity of severe acute respiratory syndrome coronavirus 19 infections also in the pediatric age as well.
- Multisystem Inflammatory Syndrome in Children (MIS-C) requires a long hospital stay.

What Is New

- Longitudinal body composition on MIS-C children shows a full recovery of growth, with a significant increase in fat mass index and anthropometric parameters 6 months after the acute event.
- A reduction in physical activity and sedentary behaviors are not recommended in patients who have had a long hospitalization for MIS-C.

Key Words: anthropometric parameters, body composition, fat free mass, fat mass, Multisystem Inflammatory Syndrome in Children (MIS-C), skinfolds

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The ethical approval was obtained from Institutional Review Board of the hospital (protocol number 2021/ST/004). Children's parents gave their written consent for inclusion after being informed about the nature of the study.

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Since November 2020, when the World Health Organization declared coronavirus disease 2019 (COVID-19) a pandemic, the clinical characteristics of patients infected with COVID-19 infection have been described (1). Acute COVID-19 infection has been shown to have an impact on sarcopenia in the adult population, for which a multidisciplinary approach including nutritional support is required to avoid long-term COVID-19 syndrome (2–4). On the other hand, body composition may also influence the severity of acute COVID-19 disease and the persistence of functional impairment (5). Several studies have focused on the association between body mass index (BMI) and the course of COVID-19 disease, considering BMI as a surrogate for body composition (6). Data collected on pediatric and adult population reported that malnutrition and obesity were associated with higher harshness of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, elevated mortality rates, and need for invasive mechanical ventilation (7).

COVID-19 disease is more severe in malnourished children under 5 years of age. In addition, malnourished children over 5 years of age are more likely to develop severe infection cases than their peers with no history of malnutrition (8).

Current meta-analyses reporting the association between childhood obesity and worse prognosis of SARS-CoV-2 infection are in line with studies showing a correlation between BMI greater than or equal to 35 kg/m^2 and the need for invasive mechanical ventilation in adults with COVID-19 disease (9,10). Further studies are needed to clarify the mechanisms whereby obesity contributes to the severity of pediatric COVID-19 infection (11).

Since April 2020, a new severe manifestation of SARS-CoV-2 infection, called Multisystem Inflammatory Syndrome in Children (MIS-C), has been described (12,13). According to the Centers of Diseases Control and Prevention (CDC), MIS-C affects patients from 2 to 18 years of age, with ongoing or recent SARS-CoV-2 infection, presenting with fever for at least 24 hours, laboratory evidence of inflammation, and the involvement of at least 2 organs (cardiac, renal, respiratory, hematological, gastrointestinal, dermatological, or neurological) (14). A multidisciplinary evaluation at 6 months showed muscle weakness, reduced exercise capacity, anxiety, and emotional difficulties. In this regard, among the malnourished and bedridden elderly, the hyper-inflammatory and highly catabolic state underlying SARS-COV-2 infection caused myofibril breakdown and muscle degradation, resulting in acute sarcopenia that has been shown to adversely affect the course of the disease (2).

Patients hospitalized for MIS-C were predominantly of healthy nutritional status. A high number of children with obesity were reported compared to a few cases of overweight and underweight ones (15,16). Comparisons with short- and long-term body composition of children with MIS-C are not currently reported, although it is well established that nutritional status influence the clinical course of the disease.

Consequently, accurate screening for nutritional risk and appropriate assessment of anthropometric measures could be crucial to the clinical management of children affected by MIS-C. In hospitalized children, timely nutritional intervention on body composition is helpful in reversing linear growth arrest, promoting tolerance to therapeutic regimens, improving quality of life, and reducing length of hospital stay (17). When no assessment was carried out, full recovery of nutritional status occurred within 6 months after discharge. The nutritional achievements contrasted with previous studies of critically ill pediatric patients. Severely burned children showed persistent long-term hypermetabolism and hypercatabolism. Studies reported a drop in lean body mass up to 9 months (18) and growth retardation up to 2 years after the burn (19).

Later studies have analyzed body composition after the acute phase of Kawasaki disease, another inflammatory disease that may result from SARS-CoV-2 infection. Despite conflicting data (20–23), monitoring the body composition of children with Kawasaki disease may be helpful in avoiding the occurrence of central obesity. Since MIS-C related to SARS-CoV-2 infection shares similarities with Kawasaki disease, we designed this study to estimate the aftereffects of the illness on body composition.

The aim of our work was to evaluate the body composition trends linked to nutritional status in children and adolescents with MIS-C diagnosis, compared to healthy children, from admission, during hospitalization, and up to 6 months after discharge.

MATERIALS AND METHODS

A prospective study was conducted among children and adolescents, without concomitant diseases, with MIS-C diagnosis according to the CDC classification (14). They were recruited at Children's Hospital V. Buzzi, in Milan, between December 2020 and February 2021. For all patients, anthropometric measurements have been recorded, on admission (T0), at discharge (T1), after 10 (T2), 30 (T3), 90 (T4) and, 180 (T5) days. Patients with moderate/severe impairment of the multiple organs, in particular cardiac function, severe hydro-electrolyte disturbance, or shock patients, required admission at the pediatric intensive care unit (PICU). During ICU care, feeding was minimal, with a few cases requiring parenteral nutrition. Physical examination including anthropometric measurements (weight, height, circumferences, skinfolds) was assessed for each patient. Weight and height before admission have been collected from a previous pediatric examination (maximum 3 months before). Weight and height, during hospitalization, were measured using a mechanical column scale with altimeter (Seca 711 and Seca 220), arm and waist circumferences (AC and WC) were measured with measuring tape (Seca 201), then AC *z* score (ACz) and WC *z* score (WCz) were calculated (24,25). Skinfolds tricipital (TSF), bicipital, subscapular, and supra-iliac were measured using a caliper (Holtain 610). The *z* score for tricipital skinfold (TSFz) were detected (26). All the anthropometric measures were collected by the same dietitian from admission at the hospital up to 180 days after dismission. BMI and BMI *z* score (BMIz) were established according to CDC growth chart reference values (27). Arm muscular area (AMA), arm fat area (AFA), and their *z* score (AMAz; AFAz) were calculated (24). Fat mass (FM), free fat mass (FFM), and indices (FMI and FFMI) were calculated according to skinfolds predictive equations for children and adolescents (28–31), at admission and dismission from the hospital and at 10, 30, 90, and 180 days after hospitalization and FMI/FFMI ratio were detected. The STRONGkids questionnaire (32), was performed during hospital stay by a dietitian to assess the risk of malnutrition. Furthermore, before discharge, nutritional advice was provided to each patient according to the principles of the Mediterranean diet, focusing on reducing sugar daily intake, on consuming whole cereals and 5 portion per day of fresh fruits/vegetables. Physical activity was limited up to 6 months after discharge, both in athletes and non-athletes children, according to the recent position statement from European Society of Cardiology (33).

Control subjects were randomly selected from the children and adolescents referring to the International Center for the Assessment of Nutritional Status (University of Milan, Italy), a nutritional outpatient clinic of the University of Milan (6,34). Children with overweight and obesity were measured at the beginning of a weight-loss program, whereas the children with normal weight were measured at the inception of a nutrition counseling program. Parents of each child gave written informed consent for the use of their child's

clinical data for research purposes. The Ethics Committee of the University of Milan approved the study procedures (study protocol No. 23/2016). We performed an individual 1:1 matching and each child with MIS-C was matched with a control child of the same sex, age class (0–3; 3–6; 6–9; 9–12; 12–15; 15–18 years), and BMI *z* score class (−1+1; +1+2; +2+3; ≥+3; −2−1; −3−2; ≤−3) at the time of discharge. This approach allowed us to understand whether the children affected by MIS-C had abnormal body composition at discharge.

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the hospital (protocol number 2021/ST/004). Children's parents gave their written consent for inclusion after being informed about the nature of the study.

Statistical Analysis

Statistical analysis was performed using STATA version 12.0 (StataCorp, College Station, TX). Continuous variables were checked for normality using the Kolmogorov-Smirnov test. As no variations from normality were observed, all continuous variables are presented as mean and standard deviation. Coarsened exact matching was used to match patients with MIS-C with control subjects on sex, age, and BMI. A *t* test for independent samples was used to test differences in outcomes of interest between patients with MIS-C and control patients. Pearson or Spearman (for dichotomous variables) correlation was used to test the association between anthropometry and body composition at hospitalization and STRONGkids with disease outcomes (length of hospitalization, day of ventilation, PICU stay). A paired *t* test was used to test changes in outcomes of interest between hospitalization and hospital discharge in patients with MIS-C. To test BMI changes during hospitalization and changes over time of the outcomes of interest from hospital discharge a repeated-measures analysis of variance , followed by a Bonferroni multicomparison test, was used. To test the effect of potential confounders (duration of hospital stay, preadmission BMI, gender, age, nutrients intake, PICU stay and time on ventilation) on anthropometrical measurements changes, mixed-effects linear regression models were used. Dichotomous confounders were categorized as follows: sex 0 = female, 1 = male; ventilation 0 = no, 1 = yes; ICU 0 = no, 1 = yes. Continuous confounders were categorized in 2 groups as follows: age 0 = <10 years, 1 = ≥10 years; preadmission BMIz 0 = −1 to +1 *z* score, 1 = ≥+1 *z* score; duration of hospitalization (on median value) 0 = <13 days, 1 = ≥13 days; energy 0 = <25th percentile of sex-for-age reference value, 1 = ≥25th percentile of sex-for-age reference value (35); carbohydrates 0 = <50%, 1 = ≥50%, sugars 0 = <15%, 1 = ≥15%, lipids 0 = <30%, 1 = ≥30%, proteins 0 = <2 g/kg body weight, 1 = ≥2g/kg body weight. To select confounders to include in the multivariate model, univariate mixed-effects linear regression models were performed. Significant confounders were included in the multivariate model. Only confounders that remained significant in the multivariate model were left. Confounders, time, and confounder × time interaction were included as fixed-effect predictors and the patient as random effect. A *P* value <0.05 was considered statistically significant.

RESULTS

Table 1 shows anthropometric parameters for all recruited children from admission to discharge. Twenty-four subjects, due to severity of symptoms, required admission in ICU. BMI *z* score significantly decreased between preadmission and hospital discharge (0.949 ± 1.121 vs 0.497 ± 1.084, $P < 0.001$) as also shown in Figure 1. Similarly, ACz (−0.457 ± 1.307 vs −0.069 ± 1.301, $P < 0.001$) and AMAz (−1.367 ± 1.403 vs −0.807 ± 1.361, $P < 0.001$) significantly decreased and AFAz (0.478 ± 0.828 vs 0.628 ± 0.856, $P < 0.001$) significantly increased between hospital admission and discharge (see Fig. 1).

Forty consecutive patients with MIS-C were enrolled in the study, 82.5% of whom were males. Patients were aged 1–17 [mean age: 9 years; standard deviation (SD) ± 4]; all subjects were normal weight for age and sex. During hospitalization STRONGkids questionnaire was performed to all children and the mean value was 3.65 (±0.67), as reported in Table 2. The average length of stay (LOS) for a hospitalization was 13.5 days (long LOS is defined when LOS ≥ 10 days (36)). Descriptive characteristics of 37 of 40 recruited patients compared to 37 healthy patients were shown in Table 2.

Groups were accurately matched according to age groups (0–3; 3–6; 6–9; 9–12; 12–15; 15–18 years), sex (the same), race/ethnicity, and BMIz (±1 SD). Sex, age, BMI, FFMI, and FMI did not differ between the 2 groups; however, AMAz was significantly lower in the MIS-C group. In Table 1, Supplemental Digital Content 1, *http://links.lww.com/MPG/D53*, we perform a correlation between anthropometry and body composition at hospitalization and STRONGkids with disease outcomes, but no associations were found.

Table 3 shows changes in anthropometric measures from discharge up to 6 months after hospitalization for all recruited patients (n = 40). BMI *z* score was significantly increased at each observation with respect to hospital dismission; the highest value was detected at T3 (BMIz at T1 0.497 ± 1.084 vs BMIz at T3 0.935 ± 1.008; $P = 0.001$) as shown in Figure 1; however, BMI *z* score at T3 did not differ from that before admission (BMIz before admission: 0.949 ± 1.121 vs BMIz at T3: 0.935 ± 1.008, $P = 0.983$). As well as ACz (−0.069 ± 1.301 vs 0.439 ± 1.092, $P = 0.001$), TSFz, instead, was significantly increased at T4 compared to baseline and with respect to the others' follow-up (0.826 ± 0.858 vs 1.093 ± 0.859, $P = 0.001$). Consistently, FMI significantly

TABLE 1. Anthropometric parameters in MIS-C patients from hospital admission to hospitaldischarge

	Pre-hospitalization		Hospitalization		Discharge		
	Mean	SD	Mean	SD	Mean	SD	*P* value*
BMIz†	0.949$_a$	1.121	0.597$_b$	1.103	0.497$_b$	1.084	**<0.001**
WCz†			1.455	1.332	1.371	1.367	0.145
ACz†			−0.457	1.307	−0.069	1.301	**<0.001**
TSFz†			0.778	0.906	0.826	0.858	0.206
WHR			0.5	0.055	0.495	0.055	0.058
FMI, kg/m^2			3.26	1.97	3.32	1.81	0.286
FFMI, kg/m^2			15.3	1.44	15.09	1.51	0.043
FMI/FFMI			0.211	0.12	0.218	0.112	0.112
AMAz†			−1.367	1.403	−0.807	1.361	**<0.001**
AFAz†			0.478	0.828	0.628	0.856	**<0.001**

Values are mean and standard deviations (SD) of anthropometric measurements and indices at different time points. Different subscript letters indicate statistically significant differences ($P < 0.05$) at Bonferroni multicomparison test. AC = arm circumference; AFA=arm fatty area; AMA = arm muscular area; BMI = body mass index; FFMI = fat free mass index; FMI = fat mass index; MIS-C = Multisystem Inflammatory Syndrome in Children; TSF = tricipital skinfold; WC = waist circumference; WHR = waist-to-height ratio. **P* values have been obtained from repeated measured ANOVA for BMI and from paired *t* test for other variables. †Values are *z* scores.

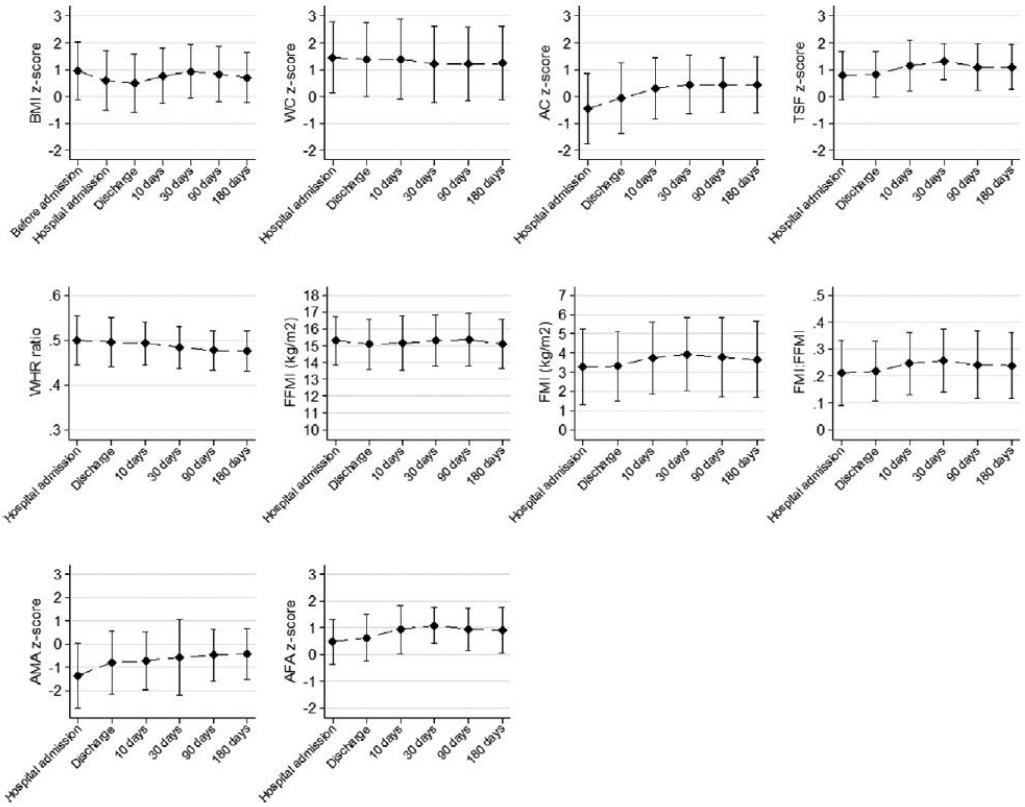

FIGURE 1. Anthropometric parameters and body composition indicex of recruited children from admission up to six months after discharge. AC *z* score =arm circumference *z* score; AFA *z* score = arm fatty area *z* score; AMA *z* score = arm muscular area *z* score; BMI *z* score = body mass index *z* score; FFMI *z* score = fat free mass index *z* score; FMI *z* score = fat mass index *z* score; TSF *z* score = tricipital skinfold *z* score; WC *z* score = waist circumference *z* score; WHR *z* score = waist-to-height ratio *z* score.

increases (see Fig. 1). FFMI at T1 was lower, then at T4 slightly increased, and at T5 remained mostly stable compared to hospital discharge (Fig. 1). FMI/FFMI ratio significantly increases, showing a peak a T2. AFAz result was significantly different between T0 and T2 (0.628 ± 0.856 vs 0.930 ± 0.912, $P = 0.001$). Moreover, we tested if changes over time of the outcomes of interest differed between patients in function of preadmission BMIz, hospitalization (LOS > 13 days), ventilation, gender, age, and nutrients intake. Regarding nutrients intake no significant associations were found. As shown in Table 2, Supplemental Digital Content 2, *http://links.lww.com/MPG/D54*, patients with BMIz preadmission ≥+1 showed a lower increment of BMIz at 6 months from discharge compared to children who were normal weight at preadmission. Children with LOS ≥ 13 days and an ICU admission with noninvasive mechanical ventilation showed a significant higher increase in WC at T4 and T5 than those who had LOS < 13 days and any ventilation, as well as waist-to-height ratio. Regarding ACz, males had a higher increment than females after discharge, and patients with a preadmission BMIz≥ +1 had a lower increment at 30 and 90 days from discharge compared to children who were normal weight at preadmission. Finally, children aged >10 years old showed lower increment of TSFz and AFAz at 10 days from discharge than younger children, but higher increment of FFMI at 3 months from discharge.

DISCUSSION

In this study we investigated the anthropometric measures and body composition longitudinally, in children and adolescents affected by MIS-C, focusing on those parameters linked to nutritional status.

We analyzed the trend between BMIz pre and after hospitalization. We also detected ACz, TSFz, FMI, and FFMI to observe trends of body composition. We found that BMIz at discharge significantly decreased with respect to preadmission values, while remaining in adequate range. At the same time, AMAz and ACz decreased and AFAz increased at discharge, suggesting a loss of lean mass and a gain in fat mass. This occurred due to reduced food intake as a consequence to diarrhea or vomiting episodes or lack of appetite. The STRONGkids questionnaires performed during hospital stay confirmed this observation, revealing a medium-to-high score of malnutrition, which might lead to a loss of metabolically active mass. Looking over the time, at T5, BMIz were respect to preadmission values, all anthropometric measures

TABLE 2. Comparison of the anthropometric measurements and indices between matched MIS-C children and control children

	Controls (n = 37)		MIS-C (n = 37)		
	Mean	SD	Mean	SD	*P* value
Sex (M/F)	30/7		30/7		1.000
Age, y	9.8	3.7	9.3	4.1	0.650
BMIz*	0.606	1.067	0.376	1.091	0.361
WCz*	0.996	1.503	1.343	1.411	0.310
ACz*	0.375	0.984	−0.022	1.108	0.108
TSFz*	0.399	1.115	0.870	0.794	**0.040**
WHR	0.46	0.06	0.48	0.05	**0.043**
FFMI	14.64	2.10	14.93	1.51	0.516
FMI	3.95	2.22	3.34	1.85	0.214
FMI:FFMI	0.26	0.12	0.22	0.11	0.103
AMAz*	0.133	0.855	−0.848	1.231	**<0.001**
AFAz*	0.423	1.032	0.697	0.785	0.204
Sk-Q			3.65	0.67	

Values are mean and standard deviations (SD) of anthropometric measurements and indices of matched MIS-C children at the time of discharge and control children. P values have been obtained from a chi square test for sex distribution and from *t* test for other variables. AC = arm circumference; AFA = arm fatty area; AMA = arm muscular area; BMI = body mass index; FFMI = fat free mass index; FMI = fat mass index; Sk-Q = STRONGkids questionnaire; TSF = tricipital skinfold; WC = waist circumference; WHR = waist-to-height ratio. *Values are *z* scores.

related to body fat accumulation, such as ACz, TSFz, AFAz, and FMI, are significantly increased. These results suggest that linear growth was restored within 6 months from the acute event, but the lean mass lost during hospitalization was not fully recovered and the fat mass has increased simultaneously. Furthermore, despite all patients having received nutritional advice to follow after hospital discharge, they were following anti-inflammatory cortisone therapy from dismission up to 10 days later, which is known to lead to hyperphagia and redistribution of lean mass and fat mass (37). Studies on adults patients, affected by SARS-CoV-19, reported that a long stay in the hospital could rise the risk of malnutrition induced by weight loss, reduced appetite, and difficulty in feeding in patients especially those with mechanical ventilation (continuous positive airways pressure mask) (38,39). In children, during the acute phase of MIS-C, metabolic and fatty acids blood profile alterations were detected (40,41), and long terms effects of malnutrition, such as a loss of lean mass, has been related to SARS-CoV-19 severity (8). In fact, even in our sample, a greater increase in WC was found both at 3 and 6 months after discharge for those who had a long hospital stay and had received noninvasive mechanical ventilation. It can therefore be hypothesized that in more severe forms of the disease there is an increased susceptibility to the accumulation of abdominal fat tissue and concomitant loss of lean mass up to several months after discharge. Moreover, among children with obesity, loss of muscle mass was highly prevalent. However, in our sample the anthropometric parameters described a normal resumption of the growth trajectory, probably due to the healthy condition (for instance, to an adequate nutritional status in most patients) and the absence of chronic illness before hospitalization in all children and adolescents enrolled. The FFMI did not seriously decline, suggesting the ability to face the acute phase of the disease, especially for male children older than 10 years. Besides, our interesting finding was that, even though patients lost muscle mass in acute phase and then they maintained FFM over time the fraction of FM increased over time, and this may be due as physical activity was suspended up to 6 months after infection. These observations led us to infer that following hospital discharge, a slow resumption of daily activities may lead to an accumulation of adipose tissue even in healthy MIS-C children. The limitation of moderate-to-vigorous intensity physical activity in the following 3–6 months that was recommended by cardiologists (33), depend on the heart damage reported by the patients, and the subsequent establishment of sedentary habits may have contributed to the excessive accumulation of fat mass. Speculating, we can say that a loss of muscular strength was probably experienced, in fact some of the patients in our sample made a subsequent admission to our hospital complaining of difficulty in walking or holding objects and most of them experiencing difficulty in climbing steps and premature fatigue overall; still some of them reported a consistent loss of hair, as a consequence of MIS-C already described in the literature (42,43), which might be linked to a poor nutritional status (44).

TABLE 3. Changes in anthropometric measurements overtime

	Discharge		10 days		30 days		90 days		180 days		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P* value*
BMIz†	0.497_{a}	1.084	$0.762_{b,c}$	1.032	0.935_{c}	1.008	$0.837_{b,c}$	1.042	$0.700_{a,b}$	0.937	**<0.001**
WCz†	1.371	1.367	1.389	1.484	1.202	1.418	1.212	1.369	1.237	1.370	0.205
ACz†	-0.069_{a}	1.301	0.297_{b}	1.131	0.439_{b}	1.092	0.435_{b}	1.013	0.419_{b}	1.041	**<0.001**
TSFz†	0.826_{a}	0.858	$1.151_{a,b}$	0.958	1.312_{b}	0.671	$1.093_{a,b}$	0.859	$1.097_{a,b}$	0.847	**0.002**
WHR	0.495_{a}	0.055	0.493_{a}	0.048	$0.484_{a,b}$	0.047	0.477_{b}	0.044	0.476_{b}	0.045	**<0.001**
FFMI (kg/m^2)	15.09	1.51	15.15	1.62	15.31	1.51	15.37	1.55	15.12	1.45	0.226
FMI (kg/m^2)	3.32_{a}	1.81	3.75_{b}	1.88	3.94_{b}	1.89	3.77_{b}	2.06	$3.67_{a,b}$	2.00	**0.002**
FMI/FFMI	0.218_{a}	0.112	0.246_{b}	0.115	0.256_{b}	0.118	$0.242_{a,b}$	0.126	$0.239_{a,b}$	0.122	**0.007**
AMAz†	−0.807	1.361	−0.738	1.256	−0.577	1.623	−0.473	1.115	−0.427	1.101	0.313
AFAz†	0.628_{a}	0.856	0.930_{b}	0.912	1.090_{b}	0.682	0.940_{b}	0.779	0.914_{b}	0.856	**<0.001**

Values are mean and standard deviations (SD) of anthropometric measurements and indices at different time points. Different subscript letters indicate statistically significant differences ($P < 0.05$) at Bonferroni multicomparison test. AC = arm circumference; AFA = arm fatty area; AMA = arm muscular area; BMI = body mass index; FFMI = fat free mass index; FMI = fat mass index; TSF = tricipital skinfold; WC = waist circumference; WHR = waist-to-height ratio. **P* values have been obtained from repeated measured ANOVA. †Values are *z* scores.

CONCLUSIONS

To the best our knowledge this is the first study to enroll a large pediatric MIS-C population and assess anthropometric measurements and body composition during acute infection and a follow-up of 6 months after discharge. The important feature identified was that the MIS-C population was comparable to healthy children before developing the syndrome and that despite the long hospital stay, they recovered their growth curve. The limitation of our study is that it was not possible to have all anthropometric measures before hospital admission except for BMIz. What is noteworthy is that reduced physical activity and limited movement, due to both medical indications and COVID-related long-term symptoms, may have had a greater negative influence on body composition to the detriment of lean mass. Therefore, in children who are affected by MIS-C and have been hospitalized for a long time, it may be useful to identify an early resumption of unstructured physical activity (ie, have a walk, cycling, dancing, playing at the park, and doing house work) alongside standard nutritional assessment and advices, in order to prevent poor nutritional and sedentary habits resulting in early excessive FM accumulation.

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4.3 Manuscript 2

“Body composition in children with Sars Cov 2 infection: short- and long-term consequences”

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Chapter 32

Body composition in children with SARS-CoV-2 infection: Short and long term consequences

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Abbreviations

ACz	**arm circumference z score**
AFAz	**arm fat area z score**
AMAz	**arm muscular area z score**
BMIz	**body mass index z score**
CT	**computed tomography**
FFM	**fat-free mass**
FFMI	**fat-free mass index**
FM	**fat mass**
FMI	**fat mass index**
ICU	**intensive care unit**
MIS-C	**multisystem inflammatory syndrome in children**
TSFz	**tricipital skinfold z score**

Introduction

The first pediatric case of COVID-19, the disease caused by the SARS-CoV-2 virus, in the USA was reported to the Centers for Disease Control and Prevention (CDC) on March 2, 2020 (Lu et al., 2020). Since then, comprehensive clinical data collection has been initiated to determine risk factors for severe pediatric COVID-19 course and poor outcomes. Malnutrition and obesity were linked to more severe SARS-CoV-2 infections, greater mortality rates, and the requirement for invasive mechanical ventilation, according to data gathered from the pediatric and adult populations (Simonnet et al., 2020, p. 2). In the adult population, sarcopenia has been linked to acute COVID-19 infection, and to prevent long-term COVID-19 syndrome, a multidisciplinary strategy is needed, including nutritional support (Piotrowicz et al., 2021; Welch et al., 2020). In children, the severity of acute COVID-19 disease and the durability of functional impairment may also be influenced by body composition status (Ong et al., 2019; Tsankov et al., 2021). Children under five who are malnourished are subject to a more severe COVID-19 disease. Furthermore, older children who have suffered from

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malnutrition are more prone to severe infections than peers who have not experienced starvation (Kurtz et al., 2021). Studies indicating a relationship between a BMI greater than or equal to 35 kg/m^2 and the requirement for invasive mechanical ventilation in adults with COVID-19 disease are consistent with recent metaanalyses finding a link between childhood obesity and a worse prognosis of SARS-CoV-2 infection (Caussy et al., 2020; Simonnet et al., 2020). Ultimately, in approximately one of every 3000–4000 children and adolescents, a rare, severe, postinfectious hyperinflammatory condition, named multisystem inflammatory syndrome in children (MIS-C), could be developed (Nygaard et al., 2022). Below, we comprehensively review how the SARS-CoV-2 infection may affect the body composition status in children both in the immediate and long terms.

Body composition and SARS-CoV-2 during infection

Obesity, measured as body mass index (BMI) z score, was found to be among the factors that increase the risk for severe COVID-19 illness (Piotrowicz et al., 2021) in children.

Studies investigating the body composition of pediatric patients with SARS-CoV-2 are currently lacking. Several studies have noted BMI among the comorbidities, but none have assessed body composition using the available methodologies. A retrospective study by Salman et al. (Salman et al., 2022) evaluated the association between skeletal muscle mass (SMM), measured by computed tomography (CT) during hospital stay, anthropometric parameters (BMI and waist circumference), and the disease outcome in children. The enrolled population was 57 patients with a mean age of 15.6 years. Over half were Hispanic (61%), while 23% were African-American. Among recruited children, 25% (14/57) were admitted to the ICU, 21% needed intubation, and 9% died. Twenty-four patients (42%) had comorbidities including cancer ($n=10$), chronic lung disease related to prematurity ($n=4$), sickle cell disease ($n=3$), and others ($n=7$). Statistical analysis shows a significant association between comorbidities and clinical outcome (ICU admission $P=.002$, mechanical ventilation $P=.02$, and mortality $P=.01$). Multivariate logistic regression analysis for ICU admission and mortality after adjustment for comorbidities revealed that only SMM was statistically and significantly correlated. There was no statistically significant association between BMI or WC with ICU stay, mechanical ventilation, or mortality. The authors state that an increased risk of ICU admission and mortality in children with COVID-19 disease is associated with low SMM detected by CT.

A proposed mechanism for the association between low skeletal muscle mass and poor clinical course and outcome in our study is that it may result in decreased secretion of myokines or muscle-derived cytokines, which are crucial for systemic glucose metabolism. This could then result in insulin resistance, which is thought to promote defective insulin-mediated antiinflammatory response of lymphocytes (Kwon & Jeong, 2020; Villarreal-Calderón et al., 2019).

Among studies on adults with COVID-19, an increase of WC and visceral adipose tissue are linked to disease severity (Kottlors et al., 2020; Petersen et al., 2020; Watanabe et al., 2020). In this study, BMI and WC were not shown to be correlated with the clinical course and outcome. However, a systematic review and metaanalysis on the severity of COVID-19 infection and pediatric comorbidities states that children with specific comorbidities are a vulnerable population at risk for potentially life-threatening consequences of COVID-19 infection, as well as childhood obesity likely being associated with a worsened prognosis of COVID-19 infection (Tsankov et al., 2021).

Body composition and multisystem inflammatory syndrome in children (MIS-C)

MIS-C affects patients from 2 to 18 years of age, with ongoing or recent SARS-CoV-2 infection, presenting with fever for at least 24 h, laboratory evidence of inflammation, and the involvement of at least two organs (cardiac, renal, respiratory, hematological, gastrointestinal, dermatological, or neurological) (CDC, 2020).

Patients hospitalized for MIS-C were predominantly in a healthy nutritional state. However, a high number of children with obesity were reported compared to a few cases of overweight and underweight ones (Capone et al., 2020, 2021). A prompt nutritional intervention on body weight can assist hospitalized children in reversing their weight loss, increase tolerance of therapeutic regimens, and shorten their stay in the hospital (Weaver et al., 2021). Hence, proper nutritional risk screening and suitable anthropometric measure assessment may be essential to the therapeutic management of children with MIS-C (Huysentruyt et al., 2013).

Although it is well known that dietary status affects the clinical course of the disease, comparisons with short- and long-term body composition of children with MIS-C are documented. A recent study (Di Profio et al., 2023) evaluated the body composition in children who developed MIS-C during or after SARS-CoV-2 infection. Anthropometric parameters of 40 patients (82.5% male, mean age 9 years; SD ±4) were collected during hospital stay and up to 6 months after discharge. Physical examination for each participant, with a particular emphasis on those parameters related to nutritional

status, included weight, height, BMI z score, circumferences (arm and waist), and skinfolds were detected. Fat mass (FM) and fat free mass (FFM) were calculated according to skinfolds predictive equations for children and adolescents. All children have no comorbidities.

Results show that, when compared to preadmission data, BMIz at discharge dramatically dropped, while still being within acceptable limits. At discharge, arm muscular area z score (AMAz) and arm circumference z score (ACz) decreased, and simultaneously, arm fat area z score (AFAz) increased, pointing to a loss of lean mass and a gain in fat mass. This observation was supported by the STRONGkids questionnaires completed during the hospital stay, which showed a medium-to-high score of malnutrition, which could result in a loss of metabolically active mass. All anthropometric measurements associated to body fat accumulation, such as ACz, tricipital skinfold z score (TSFz), AFAz, and fat mass index (FMI), are considerably higher over time when comparing BMIz values of 6 months follow-up to preadmission values. On the contrary, fat-free mass index (FFMI) did not significantly decline, especially for males <10 years, suggesting the ability to cope with the acute stage of the illness (see Fig. 1).

These findings imply that linear growth is restored within 6 months of the acute event, however the lean mass that was lost during hospitalization was not entirely recovered, and the fat mass has simultaneously grown. Both 3 months and 6 months after discharge, a higher rise in waist circumference was observed in patients who had protracted hospital stays

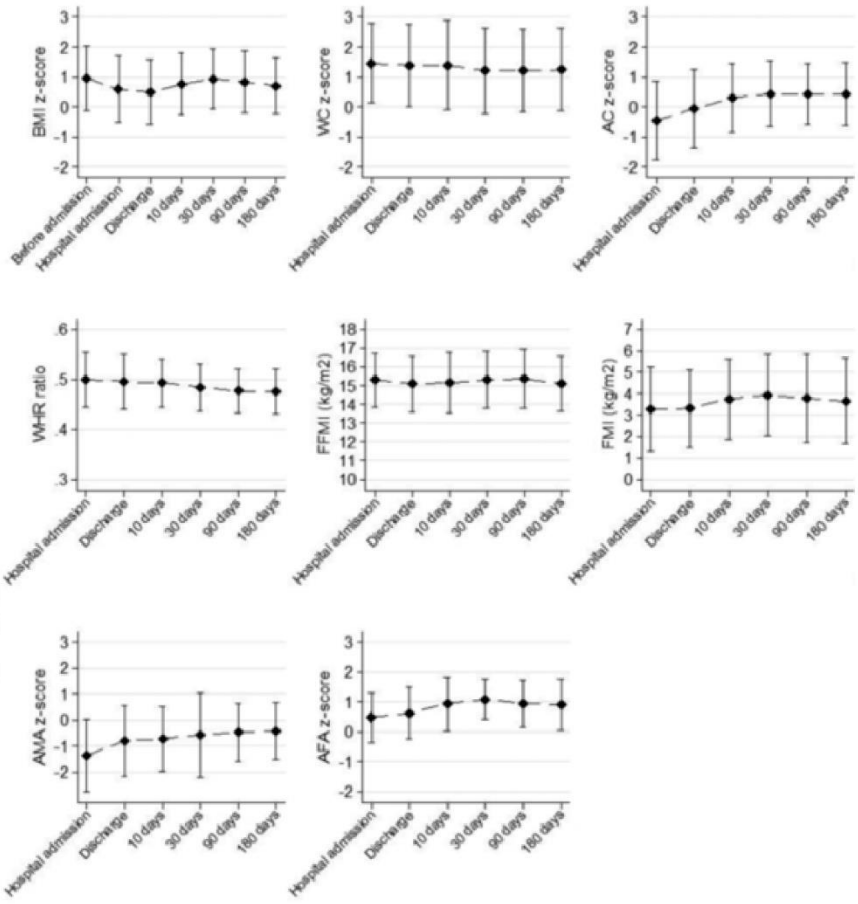

FIG. 1 Anthropometric parameters in MIS-C patients from hospital admission up to 6 months follow-up.

experiencing noninvasive mechanical ventilation. Moreover, among children with obesity, loss of muscle mass was highly prevalent, underlining a possible risk of sarcopenia.

Furthermore, MIS-C and COVID-19 both exhibit inflammatory markers, from laboratory evidence. It has been proposed that viral replication impacts the generation and release of inflammatory mediators, resulting in a severe inflammatory response in both MIS-C and COVID-19 (Nakra et al., 2020). Patients with severe COVID-19 frequently have elevated levels of cytokines, such as IL-6, as well as elevated levels of CRP and D-dimer (Malik et al., 2021; Zhou, Yu, et al., 2020). The most severe forms of COVID-19 have been attributed to an uncontrolled inflammatory response. The oxygenated metabolites derived from ω-6 PUFA may take part in both the propagation and resolution of the inflammatory response (Mazzocchi et al., 2021), but they primarily exert potent proinflammatory and prothrombotic activities. Lipid mediators, along with cytokines, are crucial in the physiological evolution of the acute inflammatory reaction.

Alterations in children's metabolic and fatty acid blood profiles were found during the acute phase of MIS-C, and long-term effects of malnutrition, like a loss of lean mass, have been linked to the severity of SARS-CoV 19 (Verduci et al., 2021).

Therefore, it can be hypothesized that there is an increased sensitivity to the storage of abdominal fat tissue and concurrent loss of lean mass up to several months after discharge in more severe manifestations of the disease.

Hence, even in healthy children who suffered from MIS-C and underwent long hospital stays, higher BMI z scores, limited physical activity, and sedentary lifestyle resulted in an accumulation of fat tissue up to several months after the acute event.

Behavioral changes during the pandemic and long term consequences

Some of the main consequences of the pandemic restrictions, such as social estrangement, the closure of educational activities in attendance, and the inability to engage in structured physical activity, influenced lifestyle and eating behaviors related to the general health of children and their families (Ammar et al., 2020). Children's social estrangement increased feelings of boredom and stress, increased energy-dense food intake and emotional eating, while reducing participation in structured physical activities and increasing sedentariness.

The resulting disproportion between energy intake and expenditure inevitably results in a positive energy balance, with increased adipose tissue deposition and weight gain (Karatzi et al., 2021).

Key drivers (diet, physical activity, sleep) affecting long-term body composition

Several studies have evaluated the indirect effects of the pandemic on children's body composition (Zemrani et al., 2021).

In a German study of 25 pupils, a significant increase in body weight and a significant, but not statistically significant, increase in BMI were observed, especially in females. This effect is not only due to general growth, but to an increase in fat mass and a decrease in muscle mass. The fat mass almost doubled after 18 months while the muscle mass decreased (Muhmann et al., 2022).

In an observational study, 220 children, including underweight, normal weight, overweight, and obese, underwent height and body composition measurements using bioimpedance analysis (BIA). Height, body mass index z score (BMIz), and muscle-to-fat ratio z score (MFRz) were calculated. This study showed that MFR z scores were significantly increased in underweight and normal weight subjects, but not in overweight and obese subjects, suggesting sedentary behavior affected the deterioration of body composition in children who already had excess weight (Azoulay et al., 2021).

A longitudinal study, conducted on approximately 2000 children aged 6–12 years, from 2017 to 2020, also reports an increase in the BMI z score during the pandemic. Children's height and weight were collected and BMI z scores were calculated each year. Through a linear regression, the annual change in BMI z score before the pandemic year (i.e., 2017–19) and during the pandemic year (i.e., 2019–20) was then estimated. Analysis showed that, before the pandemic, the annual change in children's BMI z score was +0.03 while the change during the pandemic was +0.34 (Weaver et al., 2021).

In a retrospective study, a cohort of almost 20,000 children and adolescents aged 5–17 years was examined to assess pandemic-related weight changes. BMI during the 2020 pandemic and BMI during the same period in 2019 were compared. The authors found that young people gained weight during the pandemic and the greatest change in BMI occurred among school-aged children compared to adolescents. Overweight and obesity also increased among children aged 5–11 years from 36.2% to 45.7% during the pandemic (Woolford et al., 2021).

Changes in food habits, physical activity, and mental health, and sleep disturbance have been widely documented among the pediatric population (Scapaticci et al., 2022).

Dietary habits

One of the factors that may have contributed to the increase in body weight in children during the pandemic may be changes in eating habits. Significant increases in the frequency of intake of sugary drinks, sweets, and desserts were observed during the isolation (Cipolla et al., 2021).

An Italian survey conducted during 3 weeks of home isolation included 41 obese children aged between 6 and 18 years and showed that the intake of chips, red meat, and sugary drinks increased significantly during confinement (Pietrobelli et al., 2020).

Censi et al. conducted an online survey on eating habits and physical activity of 1027 Italian children aged between 2 and 11 years during lockdown (Censi et al., 2022). This study showed that only 32.3% of the children had a high adherence to the Mediterranean diet, with better scores in children aged 2–5 years (Censi et al., 2022).

An Italian pilot study of 500 participants aged between 5 and 14 years reported that the basal body weight of 59.7% of children and adolescents increased after the pandemic. Specifically, 16.2% of the samples increased their body weight by more than 3 kg, with no significant differences between the genders. Furthermore, the authors found a greater weight gain in adolescents than in children. Lastly, a subgroup analysis, after excluding obese subjects, did not find different results, suggesting that changes in dietary habits also had an effect on the portion of the pediatric population assumed to have a healthier dietary pattern (Pujia et al., 2021).

One of the largest studies is the NutriNet-Sante cohort, which surveyed a population of 40,000 French adults and families between March and May 2020 (Deschasaux-Tanguy et al., 2021). The results suggest that home confinement has favored, in a substantial part of the population, unhealthy nutritional and lifestyle behaviors such as decreased physical activity (53%), increased sedentariness (63%), increased snacking (21%), decreased consumption of fresh food (27%), increased consumption of sweets (22%), and eating in response to boredom (18%) or anxiety (10%), with an average weight gain of 1.8 kg for 35% of respondents. The most worrying finding reported by the authors is the persistence of most of these bad habits, also in the pediatric population, more than 3 months after the lockdown: 20% of the children continued to consume unhealthy snacks and 37% of them maintained the habit of spending a lot of time in front of screens (Deschasaux-Tanguy et al., 2021).

Physical activity and sedentariness

Dunton et al. evaluated the effects of the pandemic on the physical activity and sedentary behavior of US children, aged 5–13 years, and found that the most common physical activities practiced during the initial COVID-19 period were free play/unstructured activities (e.g., running) in 90% of children and walking in 55% of children. Parents of older children (9–13 years) perceived a greater decrease in physical activity and a greater increase in sedentariness from the period prior to the start of the COVID-19 pandemic than those of younger children (5–8 years) (Dunton et al., 2020).

Moreover, a scoping review investigated the effects of restrictions on children's physical activity and their determinants. Eighty-four studies from the USA, South America, Europe, and Oceania were included in the analysis (Rossi et al., 2021). The results showed a decrease in physical activity during the pandemic, ranging from less than 10.8 min/day to less than 91 min/day, underlining how time spent on physical activities decreased with increasing age of children and lower socioeconomic background (Rossi et al., 2021).

A systematic review, which included 15 studies from European and Asian countries of children and adolescents under the age of 18, reported that there was a significant increase in body weight, BMI, waist circumference, and body fat percentage in 59.7% of the test population during the pandemic (Karatzi et al., 2021).

A metaanalysis evaluated 12 studies, conducted in eight different countries, on populations of children and adolescents aged between 2 and 18 years, found a significant increase in body weight and BMI during the lockdown and also showed an increase in the prevalence of obesity and overweight. The authors point out that there were significant reductions in the duration of exercise and, in addition to the decrease in physical activity, children had more sedentary behavior and spent more time playing video games or watching television (Chang et al., 2021).

A cohort study of 604 children aged 2–10 years in Singapore investigated the extent of restriction-related lifestyle changes during the COVID-19 pandemic, their differences by age, and the potential association with childhood adiposity 1 year after the lockdown. Among primary school children, all measures of adiposity were higher after the lockdown. The mean BMI z score was higher than before lockdown. In preschool children, the mean BMIz and the tricipital skinfold z scores were higher compared to prepandemic values.

The elimination of outdoor play or exercise was associated with an increase in BMI z score and triceps skinfold thickness, especially in primary school children, more prevalent among low-income families (Sum et al., 2022).

Sleep

Long-term isolation, anxiety, contracting an infection, annoyance and boredom, isolation from peers and teachers, and a lack of space at home are only a few of the variables affecting children and adolescents psychologically during the pandemic. Quarantine for COVID-19 was associated with tedium, tension, impatience, annoyance, and a variety of neuropsychiatric symptoms. When compared to the prior year, there was a documented 11.6% rise in the use of mental medications in the pediatric population during the pandemic. Among Chinese adolescents aged 12–18, a high prevalence of symptoms of depression (43%), anxiety (37%), and mixed anxiety and depression (31%) during the COVID-19 outbreak (Zhou, Zhang, et al., 2020), as well as among US adolescents (Racine et al., 2020), was documented.

Sleep disturbances that occurred during the SARS-CoV-2 pandemic could also have a major impact on emotional health and immune function, affecting regulation of hunger/satiety. Inadequate sleep increases the risk of cardiometabolic illness in both kids and teenagers and causes worry or mood swings, which may have been made worse by poor mental health during the COVID-19 pandemic (Bates et al., 2020). On the other hand, excessive sleep, which Pietrobelli et al. found to be an increase of 0.65 h per day during lockdown among Italian children compared to data from 2019 (Pietrobelli et al., 2020), may also affect the circadian system and influencing body composition. The increased screen time also has a detrimental effect on the quality of sleep since blue light from device screens near bedtime can inhibit melatonin secretion (Bates et al., 2020).

Conclusion

Overall, these studies indicate a negative effect of COVID-19 restrictions on the eating behavior of children and adolescents, with a significant increase in time spent sitting, unhealthy eating, and a significant reduction in time spent on physical activities (Ammar et al., 2020). This led to increases in body weight and abdominal fat accumulation with significant increases in the BMI of children and adolescents during the pandemic as well (Karatzi et al., 2021). The negative effects of the pandemic seem to be more pronounced, in terms of increased BMI, especially in certain categories, such as adolescents with obesity and patients with current pathologies (Cipolla et al., 2021).

The poor diet quality, sedentary lifestyle, and sleep habits acquired during the pandemic are not easily reversible in either children and parents. Childhood and adolescence are key stages for learning healthy eating habits that will accompany us into adulthood; an inadequate diet acquired at an early age may have lifelong repercussions (Deschasaux-Tanguy et al., 2021). Some of the major consequences of the pandemic restrictions, such as social estrangement, the closure of schools, and the inability to engage in structured physical activity, have affected lifestyle and body composition (Ammar et al., 2020) See Fig. 2.

We can, therefore, conclude that the COVID-19 infection in the pediatric population during the acute phase had more negative outcomes in those subjects who already had an underlying pathology. It has been detected that children affected by MIS-C suffered significant weight loss and risk of malnutrition correlated with a long hospital stay (Di Profio et al., 2023). On the other hand, it has been observed that long term effects resulted in weight increase and fat mass among the general pediatric population due to changes in lifestyle and eating habits.

Therefore, we can state that, in the case of MIS-C, it is important to monitor nutritional status during hospital stays and to provide personalized advice for the recovery of an optimal weight condition or to reduce the risk of fat mass accumulation, together with the resumption of physical activities at the earliest possible time. In the general population, on the other hand, it is good to monitor lifestyle changes and if necessary to intervene by providing advice on healthy and correct eating habits and on reducing sedentary activities (such as reducing time spent in front of screens) in order to avoid the onset of inactive lifestyles from childhood onwards.

Summary points

- Childhood obesity is probably related with a worsening prognosis of COVID-19 infection, and children with certain comorbidities are a susceptible population at risk for potentially life-threatening outcomes of COVID-19 infection.
- Children who developed MIS-C can have consequences on body composition both in the short term, as the period of hospitalization is prolonged and exposes one to the risk of malnutrition, but also in the long term, as resumption of daily activities and sports takes some time.

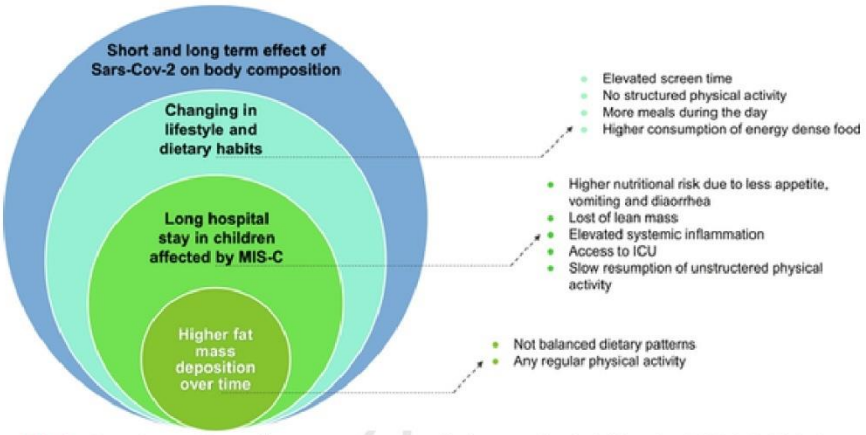

FIG. 2 Determinants of short and long term consequences of body composition in children after SARS-CoV-2 infection.

- MIS-C children should be assessed for the risk of malnutrition during hospitalization, and later should monitored to prevent fat mass storage and to promote the resumption of an adequate level of physical activity.
- Among the pediatric population, lifestyles and eating habits changed during the pandemic, as well as the level of sedentariness. The greatest weight gain was observed among adolescents.
- Many sedentary habits have been established in the everyday life of children. Such habits could have several long-term repercussions on body composition and consequently on overall health.

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4.4 Discussion and conclusion:

The poor diet and sedentary lifestyle acquired during the pandemic may not be easily reversible for either children or their parents. Since childhood and adolescence are a crucial period for learning healthy eating habits that will accompany us into adulthood, poor nutrition acquired at an early age can have lifelong repercussions [37]. In fact, Panthi et al. reported that nutrition is intrinsically linked to the immune system and susceptibility to disease, and that incorrect and unbalanced diets have serious health costs and lead to the development of chronic diseases [117].

Some of the main consequences of the pandemic restrictions, such as social estrangement, schooling online, and the inability to engage in organized physical activity, influenced the lifestyle and eating behaviours and thus the general health of children and their families [118]. Children's social estrangement increased feelings of boredom and stress, increased the intake of energy-dense foods and emotional eating, while reducing participation in organized physical activities, which led to higher sedentariness. The resulting disproportion between energy intake and energy expenditure inevitably results in a positive energy balance with increased adipose tissue deposition and weight gain [119].

A retrospective study, conducted in children under the age of 19, assessed the association between body composition measurements in children with COVID-19 admitted to hospital and the severity of the disease course and clinical outcome. The study found that lower skeletal muscle mass is associated with an increased risk of admission to intensive care and mortality in children with COVID-19, and that this effect remains statistically significant even after adjustment for the presence of comorbidities [120].

Another factor that likely contributed to the increase in body weight in children during the pandemic were changes in eating habits. Significant increases in the frequency of the intake of sugary drinks, sweets and desserts were observed during the restrictions [121]. In a retrospective study, a cohort of approximately 192,000 children aged 5-17 years was examined to assess pandemic-related weight changes. The BMI were compared before and during the 2020 . The authors found that young people gained weight during the

pandemic. The greatest change in BMI occurred among children aged 5-11 years, with an increase in BMI of 1.57, compared to 0.91 among 12–15-year-olds and 0.48 among 16–17-year-olds. Considering height this translates into an average increase of 2.30 kg more among 5–11-year-olds during the pandemic compared to the reference period, 2.31 kg more among 12–15-year-olds and 1.03 kg more among 16-17 year olds.

Overweight and obesity also increased among children aged 5-11 years from 36.2% to 45.7% during the pandemic[122]. A cohort study of 604 children aged 2-10 years in Singapore investigated the extent of restriction-related lifestyle changes during the pandemic, their differences by age, and the potential association with childhood adiposity one year after the lockdown. Among primary school children, all values of adiposity were higher after the restrictions. The mean BMIz score was 0.57 higher than the pre-quarantine values (0.30 ; $p < .001$). In pre-school children, the mean BMIz score after quarantine was 0.07 higher. After lockdown, the triceps plica z score had increased compared to pre-pandemic values, but not the subscapularis z score. The lack of outdoor play or exercise was associated with an increase in BMI (0.47; 95%CI, 0.11-0.83; P= .01), BMI z score (0.2; 95%CI, 0.01-0.38; P= .04) and triceps skinfold in primary school children [123].

A systematic review, which included 10 studies on children under the age of 18, reports that there was a significant increase in body weight, BMI, waist circumference and body fat percentage in 59.7% of the test population during the pandemic [119]. A meta-analysis including 12 studies, conducted in eight different countries on populations of children aged between 2 and 18 years, found a significant increase in body weight and BMI during lockdown and also an increase in the prevalence of obesity and overweight. The authors highlighted that there were significant reductions in the duration of physical activity, the children had a more sedentary behaviour, and spent more time than in pre-lockdown playing video games or watching television [124]. This effect is not only due to general growth, but to an increase in fat mass and a decrease in muscle mass. Fat mass almost doubled after 18 months, while muscle mass decreased [125].

Overall, these studies indicate the negative effect of restrictions on the eating behaviour of children and adolescents, with a significant increase in time spent sitting, unhealthy eating, and a significant reduction in time spent on physical activity [118]. This led to increases in body weight and abdominal fat accumulation with significant increases also in the BMI of children and adolescents during the pandemic. [119]. The negative effects of the pandemic seem to be more pronounced, in terms of increased BMI, especially in certain categories, such as adolescents who were already suffering from overweight and obesity [121].

Chapter 5

Glucose-insulin metabolism, blood fatty acids profile and Omega-3 Index in the acute phase of MIS-C

Fatty acids and inflammatory markers after DHA supplementation in MIS-C children

5.1 Introduction:

The effects of the SARS-CoV-2 infection in children and adolescents differ from those seen in adults. Young individuals generally encounter minor symptoms; however, a very low percentage of these children experience severe symptoms. Such symptoms are more common when concomitant conditions such as diabetes, obesity, or cardiovascular disorders are present. It seems that the infection causes T-helper cell stimulation and macrophage activation. As a result, cytokines are released, neutrophils, macrophages, and monocytes are stimulated, and B-cells and plasma cells are activated, producing antibodies that trigger an overreaction to the immune system[11]. This immune deregulation is associated with MIS-C syndrome in these children.

The presence of hyperglycaemia is frequently observed in critically ill non-diabetic children and is a stress response caused by high levels of peripheral insulin resistance (IR), relative insulin deficiency, and impaired glucose metabolism[62,126]. The effects of medications such as catecholamine, glucocorticoids, and exogenous dextrose administration should also be considered [126]. Although strict glucose management is not associated with a significant reduction in hospital mortality [64], blood glucose fluctuation is associated with multiorgan dysfunction [127]. Moreover, prolonged hyperglycaemia is an independent risk associated with death [63] .

There is evidence that lipid mediators may be important in the process leading to the physiological resolution of the inflammatory process, during which a new genera of metabolites formed from ω-3 PUFA was shown to have strong anti-inflammatory and pro-resolution qualities [127] . In fact, ω-3 PUFA influences immunity and initiates inflammatory resolution mechanisms [111]. Although oxygenated metabolites produced from ω-6 may play a role in the initiation and resolution of the inflammatory response, their primary effects are thrombotic and pro-inflammatory. On the other hand, specific pro-resolving mediators (SMPs) can be generated from ω-3 PUFAs. Specifically, SMPs are byproducts of lipoxygenase-mediated processes that begin with ω-3 PUFAs DHA and EPA. SMPs can encourage the physiological resolution of the inflammatory process

because they are potent anti-inflammatory agents [74,128]. The O3I reflects the ratio of the total weight of FA in red blood cell membranes to the ω-3 EPA and DHA content of red blood cells [129]. Higher levels of circulating PUFAs, particularly ω-3 PUFAs, have been associated with a better prognosis for COVID-19 in adult patients [130].

Higher DHA levels have been associated with a 26% decreased risk of hospitalization, testing positive, and death rates from COVID-19 in a large prospective population-based cohort[131]. Additionally, supplementing with ω-3 improves respiratory and renal function, reduces the likelihood of a positive SARS-CoV infection test result, reduces the intensity of symptoms, and raises survival rates[132]. Furthermore, compared to individuals with a lower O3I value, those with an O3I of 5.7% or higher had a roughly 75% lower probability of mortality. There is a distinct pattern that suggests a potential relationship; however, this variation in the risk of death is not statistically significant[104].

Taken together, the above findings support the idea that EPA and DHA have anti-inflammatory properties that could contribute to reducing morbidity and mortality in SARS-CoV-2 infection. In children ω-3 supplementation is currently used for inborn metabolic diseases and inflammation-based diseases. EPA and DHA have been used to treat obesity, hypercholesterolaemia, and NAFLD [87,89,91–93] . In MIS-C children ω-3 supplementation has not yet been investigated.

5.2 Manuscript 1

"Impaired Glucose-Insulin Metabolism in Multisystem Inflammatory Syndrome Related to SARS-CoV-2 in Children"

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Communication

Impaired Glucose-Insulin Metabolism in Multisystem Inflammatory Syndrome Related to SARS-CoV-2 in Children

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Abstract: An interaction between metabolic glucose impairment and coronavirus disease 2019 is reported. The development of a severe multisystem inflammatory syndrome in children (MIS-C) related to SARS-CoV-2 infection has been described. We evaluated the impact of MIS-C on glycemic patterns in pediatric patients. A group of 30 children and adolescents affected by MIS-C were considered; all patients were normal weight. Clinical and biochemical assessments, including surrogate markers of insulin resistance (IR) such as homeostasis model analysis-IR (HOMA-IR) and triglyceride–glucose (TyG) indexes, were recorded. Patients were also invited to undergo an intermittently scanned continuous glucose monitoring (isCGM). HOMA-IR index was calculated in 18 patients (60%), of which 17 (94%) revealed a pathological value. TyG index was computed for all patients and pathological values were detected in all cases. In 15 patients, isCGM data were recorded on average for 9 days (±3 days). Overall, average glucose was 105 mg/dL (±16 mg/dL) and average time spent in the 70–180 mg/dL range (TIR) was 93.76%, with nearly 10% of glucose readings in the 141–180 mg/dL range; glycemic fluctuations over the hyperglycemic threshold were detected in four patients. Regular glucose monitoring may be useful to prevent metabolic imbalance and obtain a better outcome.

Keywords: multisystem inflammatory syndrome; SARS-CoV-2; glucose; insulin; children

1. Introduction

The literature supports an active interaction between metabolic glucose impairment and coronavirus disease 2019 (COVID-19) [1]. As reported by Hoffmann, hyperglycemia and glycemic fluctuations may be caused by the inflammatory cascade associated with the attack of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on the pancreas and the potentially impaired β-cell function [2].

During the pandemic children have been less affected than adults and SARS-CoV-2 was in most cases asymptomatic or with mild symptoms [3–5]. However, the development of a severe multisystem inflammatory syndrome in children (MIS-C) related to SARS-CoV-2 infection has been reported [6–9]. As stated by the Center of Diseases Control and Prevention (CDC), the definition of MIS-C requires patients to be less than 21 years and to have evidence of either recent/current SARS-CoV-2 infection or exposure within the 4 weeks prior to the onset of symptoms, the presence of documented fever, elevated markers of inflammation, at least two signs of multisystem involvement, and finally, lack of an alternative diagnosis (e.g., bacterial sepsis, toxic shock syndrome) [10].

Hyperglycemia is a common complication in critically ill non-diabetic children and represents a stress response due to peripheral insulin resistance (IR), relative insulin deficiency, and glucose metabolism impairment [11,12]; the effects of medicaments such as catecholamine, glucocorticoids, and exogenous dextrose administration may also be considered [12]. Although tight glucose control is not related to a significant reduction in hospital mortality [13], blood glucose variability is associated with multiorgan dysfunction [14], and longer duration of hyperglycemia is an independent factor related to mortality [15].

Even though MIS-C represents a critical health condition associated with SARS-CoV-2 infection [6–9], data evaluating glucose disorders have not previously described. This exploratory study was conducted to evaluate the impact of MIS-C on glycemic patterns in pediatric patients admitted to Vittore Buzzi Children's Hospital in Milan, Italy, during the pandemic.

2. Materials and Methods

2.1. Subjects

We recruited a group of 30 Italian children and adolescents admitted between 1 November 2020 and 9 January 2021 to the Pediatric Department of Vittore Buzzi Children's Hospital (Milan, Italy) for MIS-C, defined according to the CDC classification [10]. Children with a known history of diabetes mellitus and/or insulin resistance, assumption of steroid/drug inducing hyperglycemia at admission, and suspected or proven inborn errors of metabolism were excluded.

For all patients, a clinical and biochemical assessment was recorded on admission. Moreover, patients were invited to undergo intermittently scanned continuous glucose monitoring (isCGM) through the FreeStyle Libre flash glucose monitoring (FGM) system (Abbott Diabetes Care, Alameda, CA, USA) [16]. The isCGM sensor was applied by caregivers at the back of patients' upper arm before the start of MIS-C therapy.

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the hospital (protocol number 2021/ST/004). Children's guardians gave their written consent for inclusion after being informed about the nature of the study.

2.2. Measurements and Statistical Analysis

Physical examination included anthropometric measurements of weight and height, and evaluation of the pubertal stage [17,18]. Body Mass Index (BMI) was calculated by dividing the patient's weight in kilograms by the square of the height in meters and then transformed into BMI z-scores using the WHO reference values [19]. The diagnostic procedure for confirming the MIS-C diagnosis included a complete blood count and measurements of C-reactive protein (CRP), procalcitonin, ferritin, cardiac troponin T (cTnT), N-Terminal pro-Brain Natriuretic Peptide (NT-proBNP), coagulative parameters, creatine kinase, electrolytes, and interleukin-6 (IL-6). The therapeutic protocol involved intravenous immunoglobulin and corticosteroid.

Additionally, at admission, the metabolic profile including total and HDL cholesterol, fasting plasma glucose (FPG), insulin (FPI) and triglycerides (TG) was analyzed (a blood sample was obtained in fasting state between 8:30 a.m. and 9:00 a.m.) [20].

Two indexes were used as a surrogate of insulin resistance (IR):

- Homeostasis model analysis—insulin resistance (HOMA-IR) index, defined as ([fasting plasma insulin (mU/L) × fasting plasma glucose (mg/dL)]/405) [21]; the cutoff point for pathological IR was set at the 97.5th percentile of the HOMA-IR distribution in a representative group of Italian healthy children and adolescents grouped by sex and pubertal stage [22].
- Triglyceride–glucose (TyG) index, calculated as (ln [fasting triglycerides (mg/dL) × fasting plasma glucose (mg/dL)/2]) [23,24]; the cutoff point for pathological IR was set at 7.88 [20,25].

A pairwise qualitative and quantitative analysis was performed between IR indexes and each variable (excluding FPG, FPI, and TG, since they have been used for the computation of HOMA-IR and TyG indexes). After the execution of Shapiro–Wilk's statistical tests, which revealed that a number of variables were not normally distributed, the non-parametric Spearman ρ was used to estimate the correlation between each IR index and clinical and biochemical parameters [26].

The isCGM data were investigated through the computation of the most useful metrics in clinical practice according to the Advanced Technologies and Treatments for Diabetes (ATTD) consensus recommendations [27], such as:

- Average glucose;
- Glucose standard deviation (SD);
- Time below range (TBR), i.e., the percentage of glucose readings under 70 mg/dL, which can be further divided into time slightly below range in the 54–69 mg/dL range, and time severely below range under 54 mg/dL;
- Time in range (TIR), i.e., the percentage of glucose readings in the 70–180 mg/dL range, which can be further divided into time in the 70–140 mg/dL target range (TIT), and time in the 141–180 mg/dL range;
- Time above range (TAR), i.e., the percentage of glucose readings over 180 mg/dL, which can be further divided into Time slightly above range in the 181–250 mg/dL range, and time severely above range over 250 mg/dL.

All the analyses were performed using the R system for statistical computing, version 4.0.4.

3. Results

All 30 patients were normal weight [19]; no one showed acanthosis nigricans. Participants' clinical and biochemical characteristics on admission are presented in Table 1.

Due to some missing FPI data, it was possible to calculate the HOMA-IR index for only 18 patients (60%). Among these, 17 (94%) revealed a pathological HOMA-IR value. Instead, the TyG index was computed for all patients and pathological values were detected in all cases.

Bar plots in Figures 1 and 2 display the correlation coefficients of each IR index, highlighting (in green) the relationships that result statistically significant (p-value < 0.05). Respectively, sodium has a significant correlation with HOMA-IR (p-value = 0.02), while ALT (p-value < 0.01), total cholesterol (p-value < 0.01), GGT (p-value < 0.01), TSH (p-value < 0.01), and albumin (p-value = 0.02) revealed a significant correlation with TyG index.

Overall, the average glucose value is 105 mg/dL (±16 mg/dL SD). Figure 3 shows the mean percent partition of time spent within different glucose ranges. Average TIR is 93.76% (±9.94% SD) with 9.42% (±14.86% SD) of glucose readings in the 141–180 mg/dL range (dark green bar). Time spent outside the 70–180 mg/dL range is highly asymmetrical: average TBR is 5.67% (±10.19% SD), with only 0.30% (±0.64% SD) of glucose readings (orange bar) under the 54 mg/dL threshold of severe hypoglycemia, while average TAR (red bars) is 0.57% (±1.20% SD).

Table 1. Study participants' characteristics at baseline. Summary statistics are presented as frequency (percentage) or median ± interquartile range.

Variable	Summary Statistics
Sex	Female: 7 (23.33%) Male: 23 (76.67%)
Age (years)	10.68 ± 7.25
BMI (Kg/m^2)	17.70 ± 3.99
BMI z-score	0.03 ± 1.49
HbA1c (%)	5.20 ± 0.20
HbA1c (mmol/mol)	33.00 ± 2.25
FPG (mg/dL)	111.00 ± 31.00
FPI (µU/mL)	21.95 ± 11.50
TG (mg/dL)	190.00 ± 177.25
HOMA-IR index	5.15 ± 5.69
TyG index	9.20 ± 0.73
Total cholesterol (mg/dL)	118.00 ± 72.00
HDL cholesterol (mg/dL)	17.00 ± 21.00
TSH (mIU/L)	2.16 ± 1.81
GGT (IU/L)	26.50 ± 38.75
ALT (IU/L)	31.00 ± 45.50
Creatine kinase (IU/L)	68.00 ± 102.00
Albumin (g/L)	25.50 ± 7.50
Sodium (mEq/L)	132.00 ± 5.00
Potassium (mEq/L)	3.50 ± 0.90
Ferritin (µg/L)	745.00 ± 1259.25
IL-6 (ng/L)	83.00 ± 208.50
C-reactive protein (mg/dL)	236.50 ± 176.00
Procalcitonin (µg/L)	6.2 ± 11.20
NT-proBNP (ng/L)	7554.00 ± 11,143.00

BMI: Body Mass Index; HbA1c: glycated hemoglobin; FPG: fasting plasma glucose; FPI: fasting plasma insulin; TG: fasting triglycerides; HOMA-IR: homeostasis model analysis—insulin resistance index; TyG: triglyceride–glucose index; HDL cholesterol: high-density lipoprotein cholesterol; TSH: thyroid-stimulating hormone; GGT: gamma-glutamyl transferase; ALT: alanine transaminase; IL-6: interleukin-6; NT-proBNP: N-Terminal pro-Brain Natriuretic Peptide.

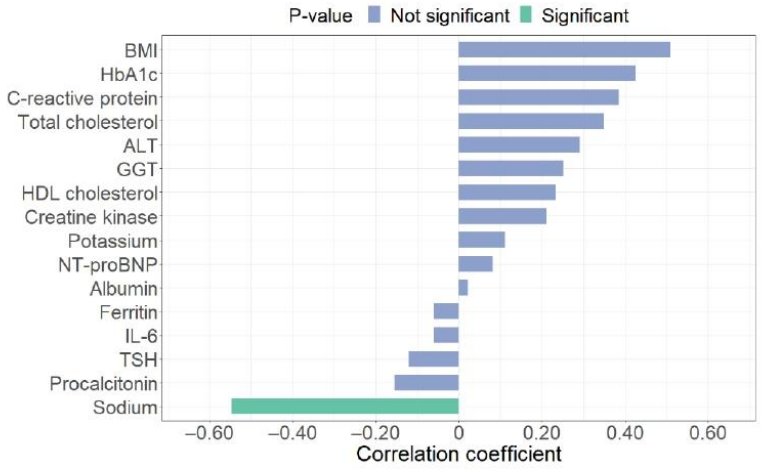

Figure 1. Spearman correlation coefficients between clinical and biochemical parameters and homeostasis model analysis—insulin resistance (HOMA-IR) index. BMI: Body Mass Index; HbA1c: glycated hemoglobin; ALT: alanine transaminase; GGT: gamma-glutamyl transferase; NT-proBNP: N-Terminal pro-Brain Natriuretic Peptide; IL-6: interleukin-6; TSH: thyroid-stimulating hormone.