Clinical Nutrition 38 (2019) 2087–2097

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Randomized Control Trials

Evidence of muscle loss delay and improvement of hyperinsulinemia and insulin resistance in Duchenne muscular dystrophy supplemented with omega-3 fatty acids: A randomized study

Maricela Rodríguez-Cruz [a, *], Salvador Atilano-Miguel [a], Lourdes Barbosa-Cortés [a], Mariela Bernabé-García [a], Tomas Almeida-Becerril [a], Alan Cárdenas-Conejo [b], Oriana del Rocío Cruz-Guzmán [a], Jorge Maldonado-Hernández [a]

[a] Laboratorio de Nutrición Molecular, Unidad de Investigación Médica en Nutrición (UIMN), Hospital de Pediatría, Centro Médico Nacional Siglo XXI (CMN-SXXI), Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[b] Departamento de Genética, Hospital de Pediatría, CMN, SXXI, IMSS, Mexico City, Mexico

ARTICLE INFO

Article history:
Received 24 April 2018
Accepted 20 October 2018

Keywords:
Duchenne muscular dystrophy
EPA
DHA
Obesity
Hyperinsulinemia
Lean mass

SUMMARY

Background & aims: Duchenne Muscular Dystrophy (DMD) is the most prevalent dystrophy of childhood and is characterized by generalized motor delays due to progressive muscular weakness, leading to loss of muscle mass. Additionally, patients with DMD develop obesity, hyperinsulinemia, and Insulin Resistance (IR). Omega-3 Long-Chain PolyUnsaturated Fatty Acids (Ω-3LCPUFA) increase fat mass, decrease lean mass, and decrease hyperinsulinemia and IR. The aim of this study was to analyze the impact of Ω-3LCPUFA consumption on lean mass, fat mass, hyperinsulinemia, and IR in children with DMD.

Methods: This placebo-controlled, double-blind, randomized study was carried out in 28 patients with DMD supplemented with 2.9 g/d of Ω-3LCPUFA ($n = 14$) or sunflower oil (placebo, $n = 14$) during 6 months. Serum glucose and insulin were measured at baseline and thereafter at months 3 and 6 of the intervention to estimate IR by HOmeostasis Model Assessment. Body composition was assessed by Dual Energy X-ray Absorptiometry.

Results: The percentage of change in EicosaPentaenoic Acid (EPA) and DocosaHexaenoic Acid (DHA) in erythrocytes was significantly ($p < 0.05$) higher in boys who consumed Ω-3LCPUFA than in the placebo group. Lean mass and fat mass (both in g/kg of Body Weight [BW]) had a trend toward being higher ($p = 0.07$ at month 3 and $p = 0.085$ at month 6) and lower ($p = 0.05$ at month 3 and $p = 0.085$ at month 6) respectively, in boys with DMD supplemented with Ω-3LCPUFA compared with the placebo group. The loss of lean mass was delayed in the Ω-3LCPUFA group; it started at month 6 but, in placebo, it started at month 3 of supplementation in comparison with the baseline of each group. Fasting insulin, percentage of boys with hyperinsulinemia, and IR were similar between the placebo and Ω-3LCPUFA groups during the 6 months of supplementation. The percentage of boys with IR was significantly ($p = 0.045$) lower at month 6 of supplementation in the Ω-3LCPUFA group than in the placebo group.

Conclusion: This study suggests that Ω-3LCPUFA (2.9 g/day) intake during 6 months likely slows the progression of muscle loss, decreases the fat mass, and reduces IR in boys with DMD. The findings of this study provide scientific background for conducting a randomized trial focused of confirming the possible beneficial role of Ω-3LCPUFA on the previously mentioned alterations mentioned in boys with early muscle damage (without fibrosis) DMD. This research was registered at clinicaltrials.gov (NCT018264229).

Abbreviations: DMD, Duchenne Muscular Dystrophy; Ω-3LCPUFA, omega-3 Long-Chain PolyUnsaturated Fatty Acids; EPA, EicosaPentaenoic Acid-20:5-3; DHA, DocosaHexaenoic Acid-22:6-3; IR, Insulin Resistance; HOMA-IR, HOmeostasis Model Assessment-Insulin Resistance.

* Corresponding author. Unidad de Investigación Médica en Nutrición, Hospital de Pediatría, Centro Médico Nacional Siglo XXI, IMSS, Av. Cuauhtémoc No. 330, Col. Doctores, Delegación Cuauhtémoc, 06725, Ciudad de México (CDMX), Mexico. Fax: +52 55 5627 6944.
E-mail address: maricela.rodriguez.cruz@gmail.com (M. Rodríguez-Cruz).

https://doi.org/10.1016/j.clnu.2018.10.017
0261-5614/

1. Introduction

Duchenne Muscular Dystrophy (DMD) is a recessive X-chromosome-linked disease that affects ~1/3600–6000 live-born males [1]. DMD appears in early childhood with generalized motor delays and gait difficulties due to progressive muscular weakness, leading to loss of ambulation by early adolescence (between 9 and 12 years of age) [2]. DMD is produced by mutations in the *DMD* gene that encodes for a sarcolemmal cytoskeletal protein called dystrophin [3–5]. Dystrophin plays an important structural role during muscle contraction. Its deficiency causes fragile muscle fiber and unstable sarcolemma, giving rise to the gradual breakdown of muscular fiber leading to loss of muscle mass, replacing the latter with fibrous connective tissue and adipose tissue, as has been reported in body composition studies, increasing adiposity and decreasing muscle mass in boys with DMD [1,6,7].

These studies have demonstrated that lean tissue mass is reduced to nearly one half the reference value of muscle mass compared with healthy boys of the same age [8]. Conversely, fat mass is elevated in subjects with DMD [7,9–11] leading to the development of overweight or obesity from the age of 7 years, reaching a frequency of >50% at 13 years of age [9]. Additionally, we reported that obesity in patients with DMD is unrelated to glucocorticoid management from the age of 7 years [12]. In patients with DMD not taking glucocorticoids, 22.7% had overweight or obesity when assessed by Body Mass Index (BMI) and, according to fat mass, a high prevalence of overweight/obesity (68%) was observed.

It is well known that obesity is associated with hyperinsulinemia, Insulin Resistance (IR), and hyperleptinemia [13]. Thus, boys with DMD entertain a high risk for developing these metabolic alterations as Carola et al., in 2017 and as we have previously reported [11,12,14]. Patients with DMD/Becker Muscular Dystrophy (BMD) present a high prevalence of IR (29%–36.4%) and hyperinsulinemia (48.5%), indicating impaired glucose metabolism. This frequency increases significantly with overweight or obesity (80%) [11,12], with a higher rate than in nondystrophic obese boys (~50% in children between 3 and 18 years of age) [15]. Consequently, hyperinsulinemia and IR add important risk factors for developing other severe morbidities, such as atherosclerosis, cardiovascular disease, and type 2 diabetes in patients with DMD [16]. In recent years, the remarkable progress in clinical and therapeutic capabilities has modified the natural history of numerous chronic disorders that initiate from childhood. As in general population, survival rates and life expectancy have also increased in patients with DMD. For that reason, the care of these patients should be improved due to that new clinical problems (metabolic disorders) have been identified [17].

The presence of metabolic alterations such as overweight, obesity, hyperinsulinemia, and IR in boys with DMD leads us to propose accurate interventions focused on halting the development of harmful outcomes. It is well known that omega-3 Long-Chain PolyUnsaturated Fatty Acids (Ω-3LCPUFA), such as EicosaPentaenoic Acid (EPA) and DocosaHexaenoic Acid (DHA), modify the body composition and decrease hyperinsulinemia and IR. It is noteworthy that there is scientific evidence provided by intervention trials with Ω-3LCPUFA in humans that have demonstrated that fat mass decreases and lean tissue mass increases following treatment in groups administered EPA and DHA than in the control group [18–20]. On the other hand, we and other authors have demonstrated that EPA and DHA decreased fasting insulin and HOMA-IR in children and adults [21,22]. Even though there is sufficient evidence on the beneficial effect of Ω-3LCPUFA on metabolic alterations, to date, it is unknown whether these fatty acids exert a positive influence on such disorders and body composition, mainly on lean tissue mass in Duchenne Muscular Dystrophy (DMD). Thus, the purpose of this study was to analyze whether supplementation with Ω-3LCPUFA might exert an impact on lean and fat mass, hyperinsulinemia, and IR in boys with DMD.

2. Materials and methods

2.1. Study design

This is a randomized, double-blind, placebo-controlled study that was registered at clinical trials.gov (ID: NCT018264229) prior to the enrollment of subjects in the study. This research was approved by the institutional ethics committee before initiation of patient recruitment (#R-2012-785-066) and was conducted at the Laboratory of the Molecular Nutrition from Medical Research Unit in Nutrition at the Mexican Institute of Social Security (IMSS). Written informed parental authorization and patients were obtained prior to study inclusion.

Selected boys with DMD were randomly assigned to the Ω-3LCPUFA (experimental) or to the placebo (control) group. The randomization scheme was planned prior to study onset by means of Random Allocation Ver. 2.0 software, by a researcher who did not participate in the measurement of outcomes, using blocks of 10 subjects. Randomization of patients included in the research was achieved using the consecutive assignment of an identification number to each patient in the interior of each stratum by a research technician, and investigators were not informed of the group assignment. When the study was concluded, the research technician disclosed the research-group assignments to the researchers.

2.2. Study population

In this study, 66 patients with a clinical diagnosis of DMD were enrolled at the Pediatrics Hospital of the CMN-Siglo XXI IMSS National Medical Center and at the “Dr. Gaudencio González Garza” General Hospital, of the CMN-La Raza” IMSS National Medical Center. From among these, 40 boys fulfilled the inclusion criteria after confirmatory molecular diagnosis of dystrophy was carried out as reported previously [23]. Patients with deletion(s) in the *DMD* gene were designated for inclusion in this study; the latter included children over 3 years of age but younger than 18 years of age, and their parents gave their written informed consent. Patients were not included if they had previously received pharmacological treatment (for instance, glucocorticoids), had ingested supplements with Ω-3LCPUFA, or had an allergic reaction to fish oil.

The study participants (Ω-3LCPUFA or placebo group) swallowed 10 identical capsules/d, equivalent to 2.9 g daily of Ω-3LCPUFA, and received doses of 45 mg, 225 mg, and 20 mg of EPA, DHA, and other Ω-3 fatty acids, respectively, per capsule (pharmaceutical grade), and molecularly distilled (Nordic Naturals, Norway) or a sunflower oil (Progela, S.A de C.V, México) as placebo during 6 months. Ω-3LCPUFA complies with the principles established for fats according to the European Pharmacopoeia Standard and according to the Council for Responsible Nutrition (CRN) and CRN-International (CRN-I). Therefore, Ω-3LCPUFA is a non-toxic supplement due to its not exceeding the maximal allowances of contaminants (peroxides, heavy metals, dioxins, and polychlorinated biphenyls). Placebo pills did not contain DHA, and only traces of EPA. In Table 1, the fatty-acid composition of the supplements is shown. Parents received indications to provide the capsules before each meal (three times a day). Also emphasized was the relevance of recording pill consumption in a diary. Capsules of Ω-3LCPUFA and placebo were identical in smell, appearance, and size, and both were strawberry-flavored to mask the taste, so that parents or patients were unable to deduce the fish-oil supplement due to subsequent eructation.

Additionally, the size of the pills (both treatments) was adequate for the participating children (they were one half of the size compared with capsules administered to adults) to improve easy swallowing for the younger patients.

After a 12-h overnight fast, morning peripheral-blood samples were obtained on a laboratory visit in a Vacutainer without and with an anticoagulant (EDTA) at baseline and monthly for the duration of the study (6 months); the samples were centrifuged at 3500 rpm. Aliquots of serum and red cells were storage at −70 °C until analysis of glucose and insulin and fatty-acids composition, respectively. After that, body fat and lean mass compartments were measured by Dual-Energy X-ray Absorptiometry (DEXA). Next, a medical history, as well as weight and height, were obtained. Subsequently, the physical capacity of the lower limbs evaluated with the Vignos scale [24] and physical rehabilitation were measured to determine, in an indirect manner, the physical activity levels of patients. Vignos scale measures the ability of the patients to move the lower-limbs, with scores ranging from 1 to 10 (walks and climbs stairs without assistance − confined to bed) [24], indicating from high to physical inactivity. Physical rehabilitation was assessed by frequency of sessions of therapies (days per week), considering that each session had a median duration of between 25 and 30 min of the stretching of upper and lower limbs. Finally a 3-day, 24-h dietary recall was applied by an interviewer, and the patients were asked not to change their dietary habits during the study.

2.3. Fat- and lean-mass measurement

Body fat and lean mass were determined by DEXA (Lunar Prodigy; GE Medical Systems, Madison, WI, USA), and enCore software Ver. 2004 (Lunar Corporation) was used to examine whole-body DEXA images. These compartments were expressed as kilograms and as g/kg of BW.

2.4. Anthropometric measurements

Qualified personnel executed measurements of BW (kg) and height (m). For subjects who were able to stand erect, height was measured with a wall-mounted stadiometer (Model 208, Seca). For non-ambulatory subjects, length was measured on a horizontal table with the boy in supine position using the summation of body-parts method [25] with a Seca tape measure. The ambulatory subject's weight was determined by means of a digital scale (Model BWB-700, Tanita), while wheelchair-bound boys were weighed on a wheelchair−bound scale (model 954, Seca) for BW wearing light-weight clothing and without shoes.

Data of Body Mass Index (BMI), expressed as percentiles, were utilized to obtain diagnoses of overweight and obesity. Boys with BMI ≤5th percentile corresponded to underweight, those with BMI >5th but <85th percentile were considered as having normal weight, BMI ≥85th but <95th percentile as overweight, and subjects with BMI ≥95th percentile, as obese, in relation to criteria established by the Centers for Disease Control and Prevention (CDC) in 2009 on BMI for children and teenagers (http://www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/about_childrens_bmi.html).

Data of height-for-age, weight-for-age, and BMI-for-age z-scores were obtained, in accordance with U. S. the Centers of Disease Control and Prevention (CDC) normative curves, by using Epi-Info (EPI-INFO 2005, release ver. 3.3.2, CDC, Atlanta, GA USA). According to BMI-for-age z-score, nutritional status was classified as follows: overweight (>1 Standard Deviation [SD]); obese, (≥2 SD); mild malnutrition (<−1 SD) and moderate malnutrition (<−2 SD).

2.5. Glucose and insulin assays for insulin resistance estimation

Glucose (mg/dL) was quantified by the glucose−oxidase enzymatic method (Glucose-LQ, SpinReact, S.A., Girona, Spain). Insulin (μU/mL) was measured using a commercial kit (Linco Research, St. Louis, MO, USA) based on radioimmunoanalysis. Hyperinsulinemia was considered if fasting insulin was >12 μU/mL, as previously reported [26]. The HOmeostasis Model Assessment−Insulin Resistance (HOMA-IR) was calculated from insulin and glucose data using the following equation: HOMA-IR = ([fasting insulin, μU/ml]* [fasting glucose, mmol/L])/22.5) [27]. A 3.16 value of HOMA-IR [28] was employed as the cut-off point for identifying boys with insulin resistance.

2.6. Compliance to supplementation procedure

Compliance with supplementation was evaluated first by the percentage % of pills consumed monthly, calculated by counting the remaining pills returned by the participants monthly. Boys taking <80% of the pills during any given month were considered non-compliant. Second, compliance was also measured (in both treatments) by analysis of EPA and DHA levels in erythrocytes at baseline and monthly during the 6 months of supplementation.

2.7. Fatty acids analysis

Fatty Acid (FA) composition was measured in red cells, as we previously reported [23], to determine EPA and DHA incorporation into tissues. Briefly, erythrocytes were removed from plasma samples by centrifugation, washed with 0.9% NaCl, and maintained at −20 °C until analysis. Total fat was extracted with isopropanol:hexane (4.5:6). Next, Fatty Acid Methyl Esters (FAME) were produced with 3N HCl in CH_3−OH and C_6H_{14} at 90 °C during 1 h. During FAME production, heptadecaenoic acid was added to all fat samples as internal standard. FAME were identified and quantified by means of gas chromatography. Identification of FAME was carried out by retention times from specific FAME standards (Poly Sciences, Niles, IL, USA). Fatty-acid results were reported as the percentage of the sum of total FA from red cells [23,29]. Fatty-acid

Table 1
Capsules fatty-acids profile.[a]

Fatty acid	Placebo % by weight	Ω-3LCPUFA % by weight
Saturated		
Lauric acid (C12:0)[b]	None	0.01
Myristic acid (C14:0)	0.07	0.29
Palmitic acid (C16:0)	6.25	2.55
Stearic acid (C18:0)	3.32	1.56
Total	9.64	4.41
Monounsaturated		
Palmitoleic acid (C16:1)	0.08	0.58
Oleic acid (C18:1)	31.31	8.88
Nervonic (C24:1)	0.06	None
Total	31.45	9.46
Polyunsaturated		
Omega-6		
Linoleic acid (C18:2)	57.71	1.58
Arachidonic acid (C20:4)	None	3.85
Total	57.71	5.43
Omega-3		
Linolenic acid (C18:3)	1.14	3.19
Eicosapentaenoic acid (C20:5)	0.07	14.46
Docosahexaenoic acid (C22:6)	None	63.04
Total	1.21	80.69

[a] 500-mg size, strawberry-flavored capsules; analysis executed at our laboratory.
[b] Fatty acid description: Number of carbons:number of double bonds.

composition was also determined in placebo and Ω-3LCPUFA pills (Table 1).

2.8. Statistical analysis

The Statistical Package for Social Sciences for Window software (SPSS ver. 24; SPSS, Inc., Chicago, IL, USA) was utilized for all analyses. Distribution was determined by the Shapiro–Wilk test. Continuous variables are described with mean values ± Standard Deviation (SD) or median (minimal, maximal), whereas qualitative variables were presented as frequency and percentages. Differences in proportions were assessed by the Fisher exact test. Comparisons between treatment groups (placebo vs. Ω-3LCPUFA) were conducted by means of the Mann–Whitney *U* test, and statistical analysis along time, by means of the Friedman Ranges Test. The relationship between Lean-Body-Mass (LBM) percentage changes and Ω-3LCPUFA (EPA + DHA) in the omega-3 group and the placebo group was evaluated by Spearman correlation coefficients. A 95% Confidence Level (95% CI) was employed, and an alpha value of $\leq$0.05 was accepted as statistically significant.

The exploratory data of the current study comprise secondary outcomes; we recently reported a randomized, double-blind, placebo-controlled trial with the aim of analyzing the influence of Ω-3LCPUFA intake on gene expression and blood inflammatory markers in boys with DMD [23]. In the present study, the impact of supplementation with these fatty acids on lean and fat mass and on hyperinsulinemia and IR were explored in the same cohort of boys with DMD. As mentioned previously, this is a secondary analysis; thus, sample size was not calculated specifically for the variables analyzed in this study. However, considering the sample size (14 subjects for each group) of this study, statistical power was computed for lean mass (g/kg of body weight) using the mean difference (614.44 vs 702.16 and 626.85 vs 713.25 at months 3 and 6 of supplementation respectively) observed between (placebo vs Ω-3LCPUFA) groups and the highest SD (132.166 and 134.97 at 3 and 6 months respectively) of a mean [30]. Statistical power obtained at 3 and 6 moths of supplementation was 77.3% and 79.70% respectively.

3. Results

3.1. Characteristics of study subjects prior to supplementation

In Fig. 1, we summarize the Consolidated Standards of Reporting Trials (CSRT) followed in this study. Forty of the 66 eligible boys decided to participate in this research and were randomly assigned to each of the treatment groups. The analysis of 28 (14 boys from Ω-3LCPUFA and 14 from placebo group) patients is presented; causes for loss-to- follow-up are also displayed in Fig. 1. Subjects

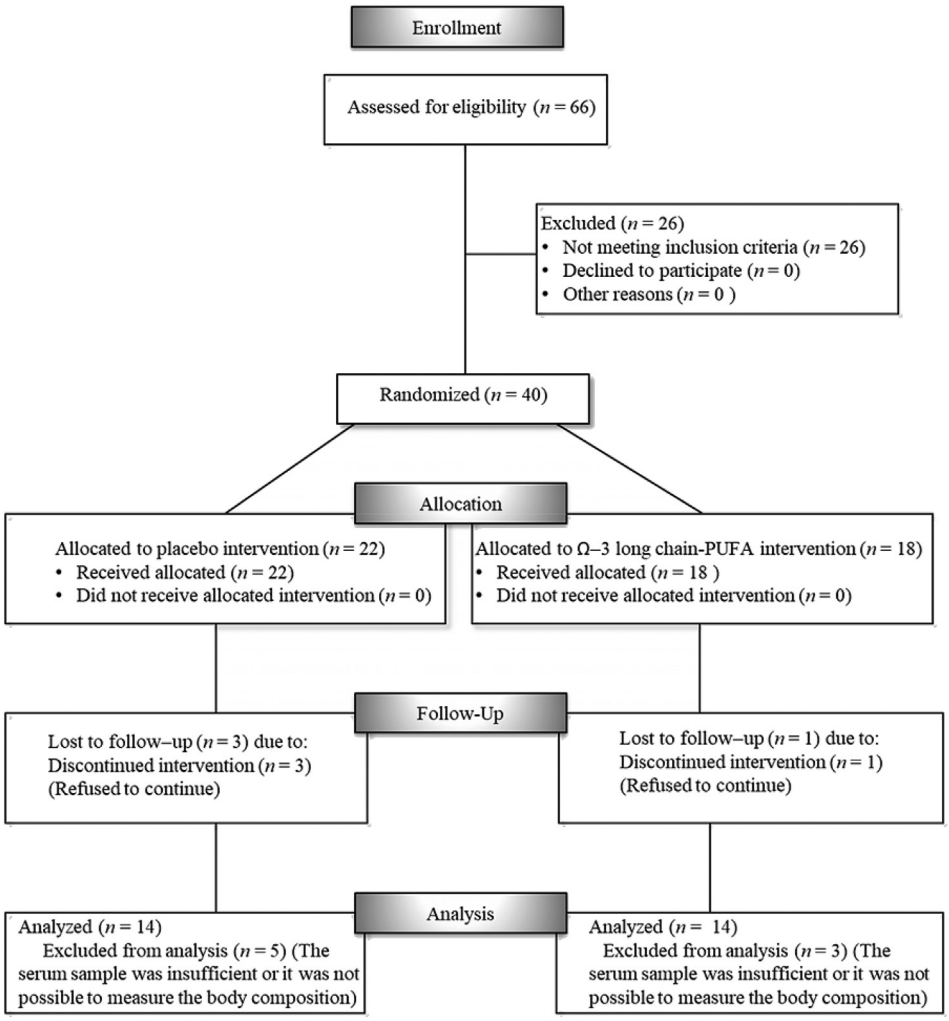

Fig. 1. Consolidated Standards of Reporting Trials (CSRT) studying patients with Duchenne Muscular Dystrophy (DMD).

were excluded for any of the following reasons; they stopped supplementation; the serum sample was insufficient, or it was not possible to measure body composition. There were no adverse effects in either supplementation group.

Age, percentage of wheelchair-bound boys, anthropometry, body composition, and the biochemical parameters of patients with DMD are indicated in Table 2.

Additionally, dietary intake measured by energy intake (kcal/kg/d), proteins, fat and carbohydrates, as well as fiber was not statistically different between groups. Finally, rehabilitation and physical capacity estimated by time and frequency of physical therapy and Vignos scale and wheelchair-bound, respectively, indicate that both supplementation groups are similar (Table 3). At baseline, there were no significant differences between groups. Also, EPA and DHA

Table 2
Anthropometry, body composition, and biochemical parameters of patients with Duchenne Muscular Dystrophy (DMD) at baseline randomly allocated to the investigation.

	Placebo ($n = 14$)	Ω-3LCPUFA ($n = 14$)	*P* value
Age (years)	8.40 ± 2.3	6.96 ± 2.5	0.129[a]
Wheelchair-bound, *n* (%)[e]	4 (28.6)	1 (7.1)	0.326[b]
Anthropometry			
Height (cm)	125.3 ± 15.2	115.3 ± 15.5	0.097[a]
BW (kg)	23.4 (16.0, 58.2)	20.2 (14.4, 58.8)	0.104[c]
Height-for-age (*z*-score)	−0.29 (−1.7, 1.6)	−0.99 (−2.51, 0.05)	0.021[c]
Weight-for-age (*z*-score)	−0.29 (−1.9, 2.8)	−0.78 (−2.8, 1.4)	0.194[c]
BMI-for-age (*z*-score)	0.45 (−2.1, 2.5)	−0.12 (−1.7, 1.8)	0.603[c]
BMI (kg/m^2)	16.6 (13.4, 28.9)	15.4 (14.0, 26.1)	0.256[c]
Overweight, *n* (%)[d,e]	2 (14.3)	4 (28.6)	0.128[b]
Obese, *n* (%)[d,e]	2 (14.3)	0	
Mild malnutrition[d,e]	1 (7.1)	2 (14.3)	
Moderately malnourished[d,e]	1 (7.1)	0	
Body composition			
Body fat mass (kg)	6.4 (2.3, 32.1)	3.3 (1.2, 31.2)	0.085[c]
Body fat mass (%)	32.2 ± 14.9	23.7 ± 13.8	0.130[a]
Lean body mass (kg)	16.3 (12.6, 28.2)	15.0 (11.2, 25.2)	0.150[c]
Biochemical parameters			
Glucose (mg/dL)	89.0 (75.0, 121.0)	82.3 (77.0, 98.0)	0.210[c]
Hyperglycemia, *n* (%)	1 (7.1)	0 (0)	0.500[b]
Insulin (μU/mL)	16.6 ± 11.1	11.3 ± 8.3	0.157
Hyperinsulinemia, *n* (% of boys)	8 (57.1)	5 (35.7)	0.449[c]
HOMA-IR	2.9 (0.7, 9.2)	1.6 (0.5, 6.1)	0.227[c]
Insulin resistance *n* (% of boys)	7 (50.0)	4 (28.6)	0.440[b]
Ω-3 LCPUFA in red blood cells			
EPA (% total fatty acids)	0.25 (0.06, 2.6)	0.7 (0.04, 2.6)	0.954[c]
DHA (% total fatty acids)	1.2 (0.11, 2.3)	1.0 (0.02, 2.6)	0.806[c]

DMD, Duchenne Muscular Dystrophy; Ω-3LCPUFA, omega-3 Long-Chain PolyUnsaturated Fatty Acids; BMI, Body Mass Index; HOMA-IR, HOmeostasis Model Assessment-Insulin Resistance.
Hyperinsulinemia was considered if fasting insulin was >12 μU/mL [25].
Insulin resistance was considered if the HOMA-IR value was >3.16 [27].
Data are presented as mean ± Standard Deviation (SD) or as median (minimal, maximal).
[a] Independent-sample Student *t* test.
[b] Pearson chi-square test.
[c] Mann-Whitney *U* test.
[d] Data are presented as number (%).
[e] Determined according the BMI *z*-score as follows: >1 Standard Deviation (SD) (overweight); ≥2 SD (obese); <−1 SD (mild malnutrition); and <−2 SD (moderate malnutrition) based on data from CDC 2009.

Table 3
Dietary intake and physical capacity and rehabilitation of boys with DMD at baseline randomly allocated to the investigation.

	Placebo ($n = 14$)	Ω-3LCPUFA ($n = 14$)	*P* value
Dietary intake[a]			
Energy (kcal/kg/d)	74.91 (22.81–128.97)	86.28 (30.6–181.87)	0.435
Carbohydrates (g/kg/d)	10.44 (3.73–12.11)	10.76 (2.52–17.6)	0.232
Fiber (g/1000 kcal)	8.01 (3.45–23.96)	11.26 (6.0–18.47)	0.089
Protein (g/kg/d)	2.74 (1.12–5.14)	3.13 (1.35–8.49)	0.491
Fat (g/kg/d)	2.32 (0.45–7.40)	2.52 (0.52–7.74)	0.520
Rehabilitation and physical capacity			
Time of physical therapy (min/session)	42.5 (0.0–60.0)	20.0 (0.0–90.0)	0.305[a]
Frequency of physical therapy (sessions/week)	7.0 (2.0–7.0)	3.0 (2.0–7.0)	0.546[a]
Vignos scale	3.0 (1.0–9.0)	2.5 (1.0–9.0)	0.646[a]
Wheelchair-bound, *n* (%)*	4 (28.6)	1 (7.1)	0.326[b]

DMD, Duchenne Muscular Dystrophy; Ω-3LCPUFA, omega-3 Long-Chain.
PolyUnsaturated Fatty Acids.
Values are expressed as median (minimal, maximal).
*Data are presented as number (%).
[a] Mann-Whitney *U* test to compare the treatments.
[b] Pearson Chi-square test.

content were similar between groups prior to the initiation of this study.

Therefore, all of these parameters, including the Ω-3LCPUFA proportion, were uniformly distributed, indicating homogeneity among subjects at the beginning of the study.

3.2. Compliance with treatments

Adherence to treatments was 95.51% ± 6.87% and 96.49% ± 5.18% mean ± SD for control and Ω-3LCPUFA, respectively. The percentage of pills ingested was similar between treatments during the 6 months (p values were 1.0, 0.603, 0.801, 0.458, 0.482, and 0.614 at months 1, 2, 3, 4, 5, and 6, respectively) of supplementation. Results revealed that the EPA and DHA percentage change from baseline increased during each month of supplementation in erythrocytes from the Ω-3LCPUFA group, and that it was higher than in the placebo group ($p < 0.05$) (Fig. 2). Taken together, these findings demonstrate acceptable compliance with treatments.

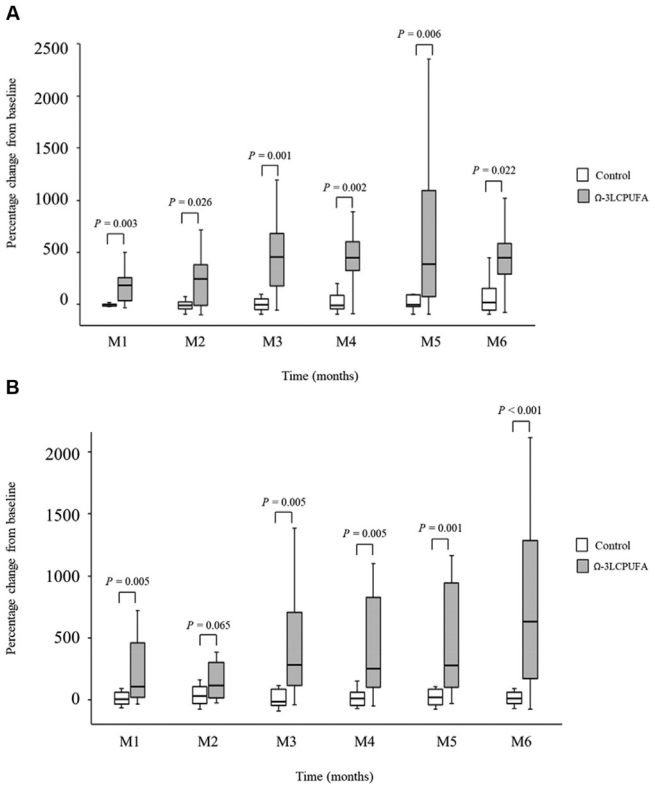

Fig. 2. Changes in the composition of erythrocyte EicosaPentaenoic Acid (EPA, 2A) and DocosaHexaenoic Acid (DHA, 2B) during the time of the study in patients with Duchenne Muscular Dystrophy (DMD).

3.3. Effect of intervention on lean and fat mass

In this study, Body Weight (BW) (kg) and BMI (percentile) were nonsignificant between groups during the 6 months of supplementation. Lean mass (g/kg of body weight) has a trend towards significance to be higher at months 3 and 6 in boys with DMD supplemented with Ω-3LCPUFA compared with the placebo group (Table 4).

Additionally, the change in the lean mass (g/kg of body weight) was directly correlated ($r = 0.670$; $p = 0.012$) to changes in Ω-3LCPUFA (sum of EPA + DHA) at month 3 (at that time, tissues were totally enriched with Ω-3LCPUFA) of supplementation with omega-3 fatty acids with respect to baseline (Fig. 3).

Although there was a loss of lean mass in both groups, this occurred earlier in the placebo group (from month 3) than in the omega-3 fatty acids group (from month 6). In Fig. 4A, it is depicted that in the placebo group, lean mass was observed since month 3 and this loss continued until month 6 of supplementation in comparison with baseline. In contrast, in the Ω-3LCPUFA group, lean-mass loss was at month 6 after initiating the study.

On the other hand, fat mass (g/kg of BW) exhibited a trend toward being significantly lower at months 3 and 6 of supplementation in boys who consumed Ω-3LCPUFA than in the placebo group (Table 4). An increase in fat mass (g/kg of BW) was observed in both study groups at month 6 of supplementation (placebo, $p = 0.035$ and Ω-3LCPUFA $p = 0.003$) with respect to the baseline of each group (Fig. 4B).

3.4. Effect of intervention on insulin and insulin resistance

In this study, there were no significant differences when comparing fasting glucose concentration between intervention groups nor during the 6 months of supplementation in each group (data not shown). Also, fasting insulin, percentage of boys with

Table 4
Anthropometry, and lean and fat mass from boys with DMD during 6 months of supplementation with placebo or with Ω-3LCPUFA.

Time after administration (months)	Control ($n = 14$)	Ω-3LCPUFA ($n = 14$)	P value[a]
BW (kg)			
Baseline	23.4 (15.9, 58.2)	20.2 (14.4, 58.8)	0.104
3	24.8 (16.4, 60.9)	19.8 (14.5, 61.6)	0.069
6	26.3 (16.8, 63.4)	21.2 (15.5, 61.5)	0.069
Percentile of BMI			
Baseline	70.9 (1.8, 99.4)	40.5 (5.0, 96.7)	0.401
3	82.2 (3.6, 99.5)	47.6 (0.5, 97.2)	0.246
6	73.6 (9.3, 99.6)	51.2 (1.4, 96.8)	0.285
Lean mass (g/kg of BW)			
Baseline	667.1 (413.3, 802.8)	768.8 (429.1, 844.6)	0.114
3	632.4 (422.3, 817.4)	762.3 (421.0, 838.9)	0.077
6	599.0 (425.6, 777.9)	752.4 (426.2, 849.9)	0.085
Fat mass (g/kg of BW)			
Baseline	276.1 (118.5, 551.9)	176.3 (86.0, 530.4)	0.104
3	310.1 (122.8, 542.7)	179.8 (95.2, 540.2)	0.050
6	338.9 (158.5, 540.3)	191.6 (88.8, 535.0)	0.085

DMD, Duchenne Muscular Dystrophy; BW, Body Weight.
Values are expressed as median (minimal, maximal).
[a] Mann-Whitney U test to compare the treatments.

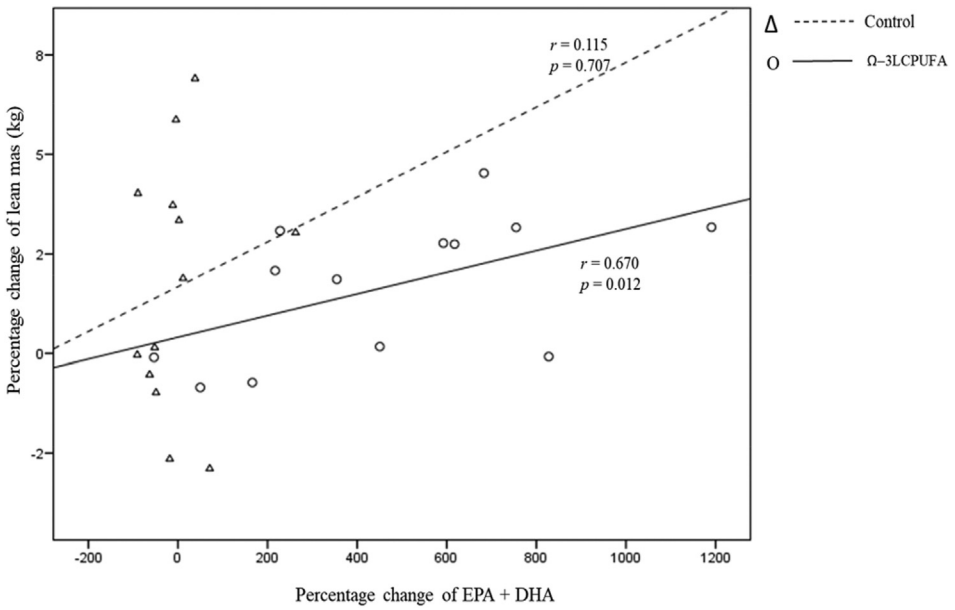

Fig. 3. Correlation between the percentage of lean-mass (kg) change and the percentage of Ω-3LCPUFA (EPA + DHA) change at month 3 from baseline in the omega-3 fatty acids and control groups. Ω-3LCPUFA, omega-3 Long-Chain PolyUnsaturated Fatty Acids; EPA, EicosaPentaenoic Acid; DHA, DocosaHexaenoic Acid.

hyperinsulinemia, and IR evaluated by HOMA-IR were similar between placebo and Ω-3LCPUFA groups during the 6 months of supplementation. However, the percentage of boys with IR was significantly ($p = 0.045$) lower at month 6 of supplementation in the Ω-3LCPUFA group than in placebo (Table 5).

There was a significant reduction in the insulin (percentage of change) at months 3 ($p = 0.019$) and 6 ($p = 0.009$) in comparison with month 1—baseline in the Ω-3LCPUFA group; in contrast, fasting insulin was not significantly affected throughout supplementation in the placebo group (Fig. 5A). In this regard, a significant reduction in the HOMA value (percentage of change) was also observed at month 3 ($p = 0.023$) and 6 ($p = 0.00$) of supplementation than month 1—baseline in patients supplemented with Ω-3LCPUFA; these differences were not observed in the placebo group (Fig. 5B).

4. Discussion

In addition to loss of muscle mass and elevated fat mass, hyperinsulinemia and IR are other complications in patients with DMD (Fig. 6) [7,9–12,14], worsening the quality of life of these patients. Therefore, in terms of generating the need to explore possible therapies to decrease these complications, it is important in palliative therapies for improving quality of life. The exploratory data of the present study suggest that treatment with Ω-3LCPUFA might delay loss of muscle mass and decrease of fat mass, hyperinsulinemia, and IR in patients with DMD, as observed in body composition and in serum fasting insulin.

The absence of dystrophin expression leads to progressive muscle weakness, with chronic degeneration of muscle and the replacement of muscle with fat and endomysial fibrosis, resulting in the loss of muscle mass (Fig. 6) [31]. Therefore, in this study, we explored the impact of supplementation with Ω-3LCPUFA on body composition as well as on lean and fat mass, and on metabolic alterations such as hyperinsulinemia and IR in patients with DMD.

First, EPA and DHA intake was examined monthly throughout the study in a sample of erythrocytes from boys with DMD from both groups (placebo and omega-3 fatty acids), describing the status of these fatty acids [32]. The increase of EPA and DHA solely in patients who consumed Ω-3LCPUFA demonstrated adequate compliance with treatments, therefore the reliability of the findings in this investigation.

The exploratory data of this study suggest that there is a tendency toward the decrease of the loss of lean mass in patients supplemented with Ω-3LCPUFA. Additionally, in this group, the loss of lean mass was observed until 6 months of supplementation. Therefore, we proposed that Ω-3LCPUFA might to slow the progression of muscle loss. This proposal is due to that in the placebo group, loss of muscle mass was observed from month 3 of supplementation. For more than 10 years, it was suggested that Ω-3LCPUFA might be used as a therapeutic agent for the management and prevention of sarcopenia [33]. Initial evidence reported in a cross-sectional and retrospective cohort study carried out in men and women aged 59–73 years. The findings of that study demonstrated an increase in grip strength in men and women for each extra portion of fatty fish (rich in EPA and DHA) ingested weekly, independent of their height and age [34]. Subsequently, in a randomized controlled trial in 16 (10 men and 6 women) older adults (≥65 years of age), it was confirmed that EPA (1.86 g/day) and DHA (1.50 g/day) act on the increase of muscle -mass and in the rate of muscle-protein synthesis [35]. In fact, other studies extensively reviewed by Ewaschik et al. (2016) reported that providing omega-3 fatty acids such as EPA and DHA as dietary supplements or such as fish oil in human diets increases anabolic potential and decreases muscle loss [36]. Furthermore, a relationship has been observed between Ω-3LCPUFA (EPA and DHA) supplementation and the conservation or increase of muscle mass in patients with cancer, who would otherwise, like the boys with DMD, experience muscle loss [37,38]. A number of mechanisms of action have been associated with the effects of Ω-3LCPUFA on muscle mass. In this respect, the results of Smith et al. (2011) indicate that one of the mechanisms might be, partially, by means of the increased activation of the mTOR-p70s6k (an integral central point for muscle-cell growth) signaling pathway [35]. Another mechanism involved is the inflammation in DMD, which is the leading driver of muscle wasting [6]. Thus, attenuation of pro-inflammatory mediators by Ω-3LCPUFA as has been reported in other diseases [21,22] and in DMD [23], might comprise another pathway for improving muscle mass in boys with DMD, and for

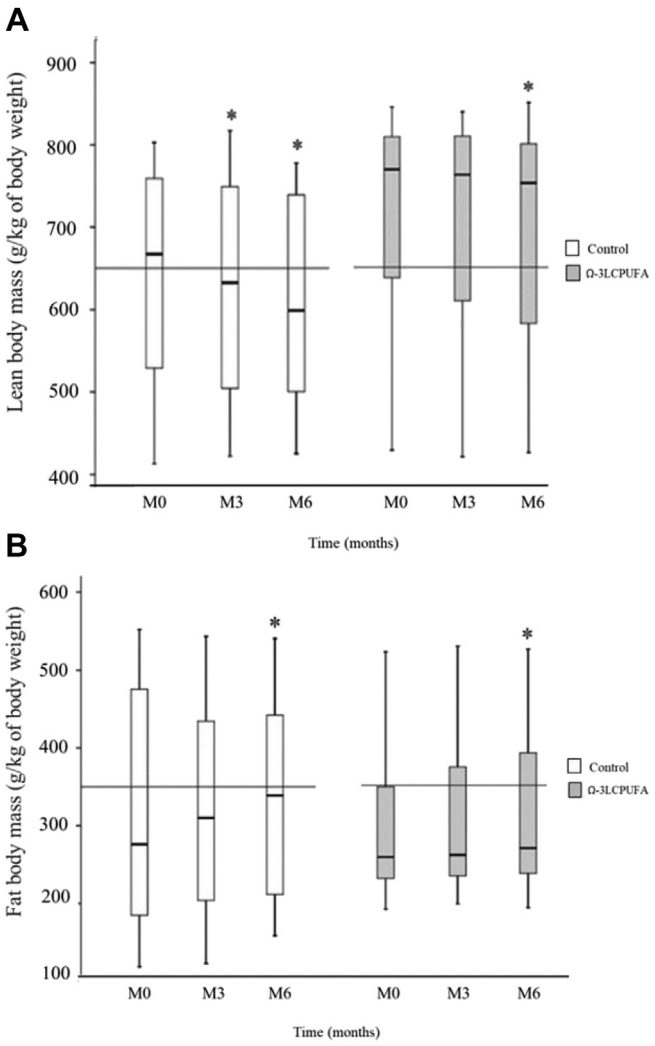

Fig. 4. Lean (4A) and fat (4B) mass (g/kg of body weight) of children with Duchenne Muscular Dystrophy (DMD) during supplementation. Values are median (minimal, maximal); *$P < 0.05$. M0 vs. M3 and M6. Statistical analysis during the time using the Friedman Range Test. Ω-3LCPUFA = omega-3 Long-Chain PolyUnsaturated Fatty Acids; M0 = baseline.

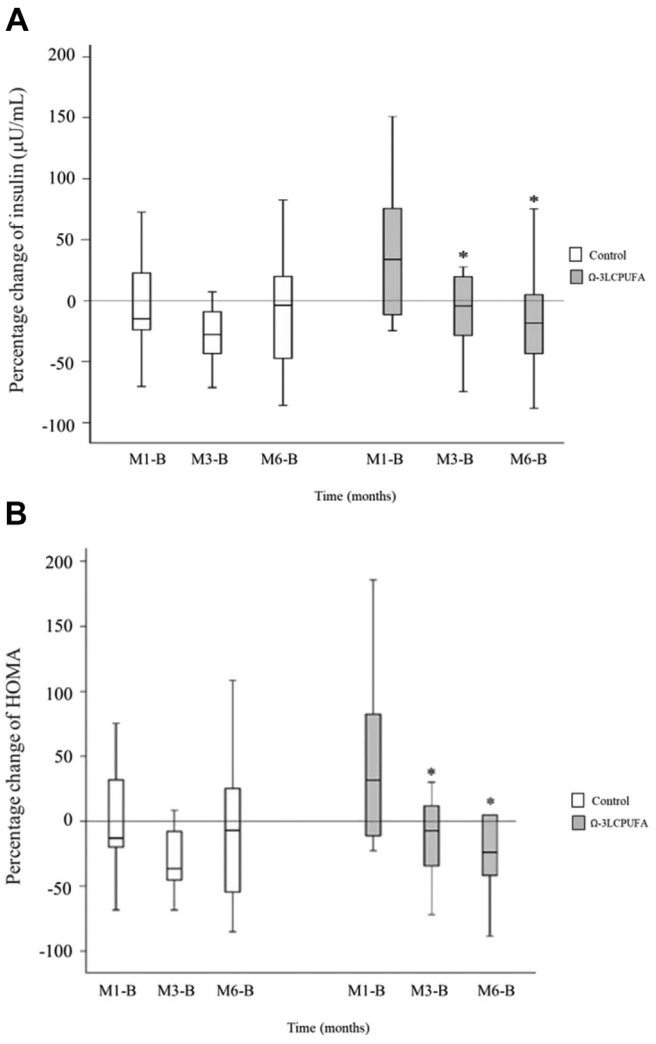

Fig. 5. Change of insulin (5A) and HOMA (5B) of children with Duchenne Muscular Dystrophy (DMD) during supplementation. Values are median (minimal, maximal); *$P < 0.05$. M1-B vs. M3-B and M6-B. Statistical analysis during the time using the Friedman Range Test. Ω-3LCPUFA = omega-3 Long-Chain PolyUnsaturated Fatty Acids. MI = Month 1, 3, or 6; B = baseline.

Table 5
Insulin and Insulin Resistance (IR) in boys with Duchenne Muscular Dystrophy (DMD) during 6 months of supplementation with placebo or omega-3 fatty acids.

Time after administration (months)	Control ($n = 14$)	Ω-3LCPUFA ($n = 14$)	*P* value
Fasting insulin (μU/mL)[a]			
Baseline	14.8 (3.0, 34.7)	7.9 (2.4, 28.4)	0.210
3	15.7 (2.3, 82.3)	5.9 (3.2, 34.1)	0.350
6	17.9 (1.5, 51.6)	6.9 (1.5, 28.9)	0.231
Fasting hyperinsulinemia n (% of boys)[b]			
Baseline	8 (57.1)	5 (35.7)	0.449
3	9 (64.3)	5 (38.5)	0.257
6	8 (66.7)	4 (28.6)	0.113
Fasting HOMA-IR[a]			
Baseline	2.9 (0.7, 9.2)	1.6 (0.5, 6.1)	0.227
3	3.2 (0.5, 15.2)	1.3 (0.7, 6.8)	0.402
6	3.9 (0.3, 15.3)	1.4 (0.3, 5.7)	0.157
Insulin resistance n (% of boys)[b]			
Baseline	7 (50)	4 (28.6)	0.440
3	7 (50)	5 (38.5)	0.830
6	8 (66.7)	3 (21.4)	0.045

DMD, Duchenne Muscular Dystrophy; HOMA-IR, HOmeostasis Model Assessment-Insulin Resistance.
Values are expressed as median (minimal, maximal).
Insulin Resistance (IR) was considered if the HOMA-IR value was >3.16 [28].
Hyperinsulinemia was considered if fasting insulin was >12 μU/mL [26].
[a] Mann–Whitney *U* test to compare the treatments.
[b] Fisher test to compare the treatments.

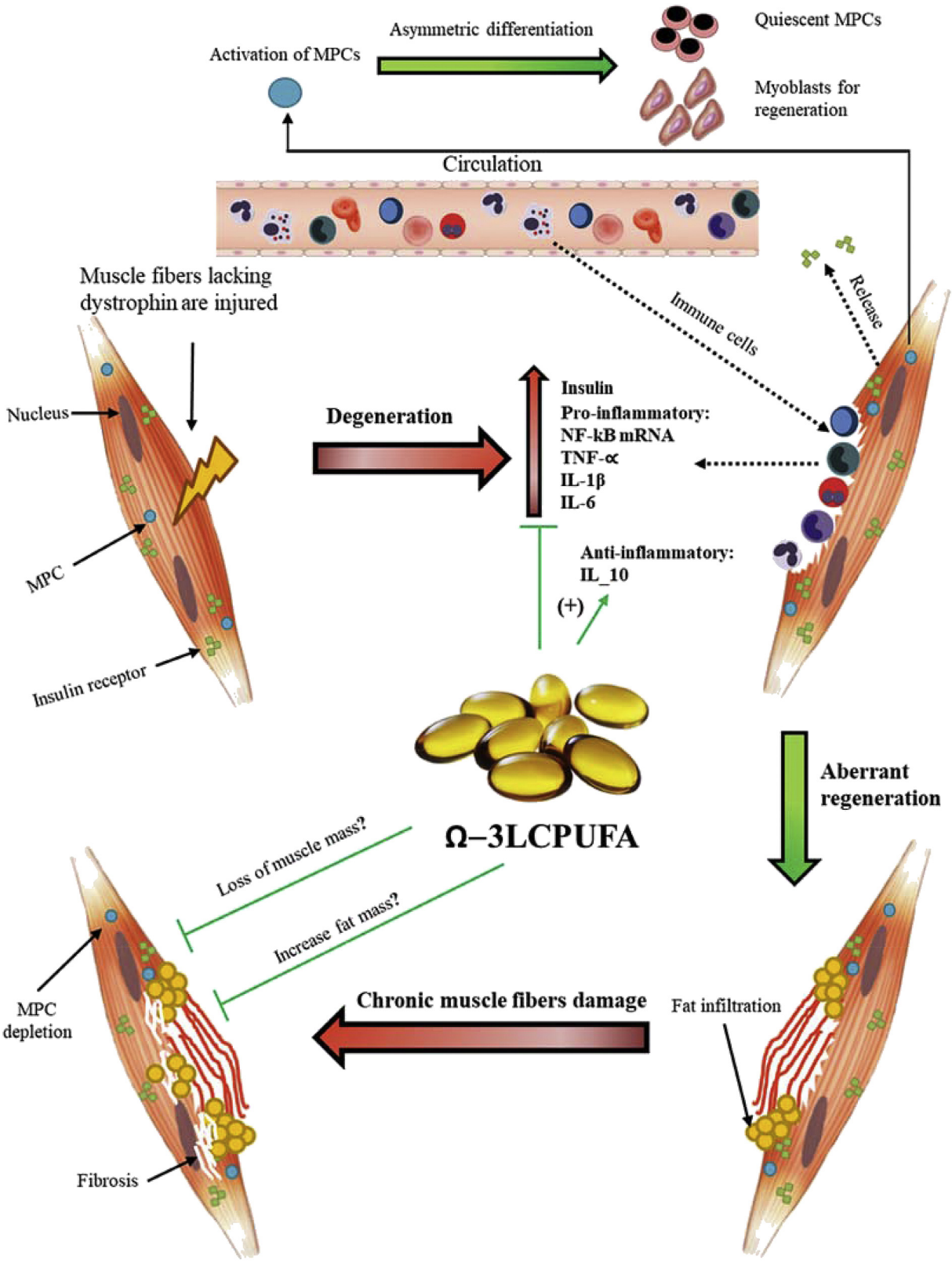

Fig. 6. The mechanism of action proposed for Ω-3LCPUFA in order for these to work collectively in concert and to attempt to recover the function of dystrophic muscle. MPC = Muscle Progenitor Cell; Ω-3LCPUFA = omega-3 Long-Chain PolyUnsaturated Fatty Acids.

preserving typical skeletal-muscle morphology, as observed in dystrophic hamsters fed a flaxseed-enriched (rich in linolenic acid, a precursor of EPA and DHA) diet [39].

Additionally, our exploratory data suggest that Ω-3LCPUFA might exert a positive effect, because they tend to decrease fat mass in boys with DMD. There is a great amount of scientific evidence provided by various animal models [36] and intervention trials that demonstrate the clinically significant impact of Ω-3LCPUFA on decreasing body-fat mass in humans [18–20]. It is likely that the lower fat mass in these boys with DMD receiving Ω-3LCPUFA may be due, in part, to that tissues (such as muscle tissue) enriched with these fatty acids reduced the master transcription factor, the Sterol Regulatory Element Binding Protein (SREBP) 1c and subsequent genes regulating downstream pathways of fatty acid synthesis [40]; thus, one of the probable mechanisms of Ω-3LCPUFA in DMD is the decrease of lipogenesis. It is well known that DHA and EPA bind directly to other master transcription factors, denominated Peroxisome Proliferator-Activated Receptors (PPAR) α and β, to activate them. These factors are abundant in skeletal muscle and other oxidative tissue and play principal roles in regulating fatty catabolism [40]. PPARα and PPARβ control the expression of several genes involved in the metabolism of fatty acids, including their transport through cell membranes, intracellular union (Fatty Acid Binding Protein [FABP]), and mitochondrial and peroxysomal β-oxidation [40]; then, Ω-3LCPUFA decrease lipogenesis and also increase fatty oxidation.

Improvement of the insulin response has been the most extensively examined mechanism utilized for assessing the beneficial effects of EPA and DHA. The findings of this study are not conclusive. Although we did not observe differences in insulin levels between treatments, in patients supplemented with Ω-3LCPUFA, a significant reduction in the insulin level and insulin resistance (evaluated by HOMA) value was observed during treatment (at months 3 and 6 of supplementation. These effects were not identified in the placebo group, suggesting the favorable effect of these fatty acids on insulin. These data are in agreement with those reported in children and adults by our team and by other authors, who observed a decrease of fasting insulin and IR after supplementation with Ω-3LCPUFA [21,22]. In the present study, the exploratory results suggest that Ω-3LCPUFA might reduce fasting insulin, which leads to diminishing the percentage of boys with IR (Table 5). The benefit of these fatty acids on the insulin response takes place in all organs of the body that depend on this hormone, including muscle tissue, as previously demonstrated by Dangardt et al. (2012) [41]. These authors observed improved insulin sensitivity and reduced triglyceride accumulation in the muscle of obese children after the administration of Ω-3LCPUFA. Therefore, with this exploratory data, we propose that EPA and DHA might similarly increase insulin sensitivity in the muscle of boys with DMD by incorporating these into muscle membrane, modifying its composition. Thus, Ω-3LCPUFA may regulate key membrane substrates that participate in the insulin signaling pathway and subsequent protein synthesis (for instance, mTOR, AKT, insulin receptor, insulin receptor substrate-1, and phosphatidylinositol 3-kinase activity) in muscle [36].

Accordingly, if Ω-3LCPUFA certainty exert a beneficial effect, they may trigger more than one response in order to work collectively in concert and to endeavor to recover the function of dystrophic muscle, as our team recently reported and as is suggested by these exploratory results of this study, reducing pro-inflammatory markers and increasing an anti-inflammatory marker [23], and possibly slowing the progression of muscle loss, in turn decreasing fat mass and diminishing fasting insulin and IR (Fig. 5).

Nevertheless, the current study has limitations. First, the findings are secondary outcomes; thus, the sample size was not estimated, and this study was carried out in the same cohort in boys with DMD from a previous research. Second, in this study it was not possible to measure intramuscular fat to evaluate disease severity in DMD, as proposed by Tishya et al. (2008) [42]. Also, we could not to determine the impact of Ω-3LCPUFA on muscle-fiber integrity. Additionally, it was not possible to obtain a muscle biopsy (prior to and at the end of supplementation) to determine whether Ω-3LCPUFA preserved the typical skeletal muscle morphology, as observed in dystrophic hamsters fed a flaxseed-enriched (rich in linolenic acid, a precursor deriving from EPA and DHA) [39]. In addition, this study included patients in a wide age range and with varying degrees of muscle function. Finally, we are unable to discard that sample size did not permit to the observance of an overwhelming impact. Despite these limitations, these exploratory data provide the scientific background on the probable potential therapeutic impact of Ω-3LCPUFA to reduce the severity of muscle loss and fat-mass decrease and the positive effect on insulin and RI in patients with DMD. Furthermore, the exploratory results of the present study can be useful in proposing a trial focused on identifying the impact of supplementation with Ω-3LCPUFA on slowing the progression of muscle loss, decreasing fat mass, and reducing the fasting insulin and IR in boys with early muscle damage (without fibrosis) reducing the age range in DMD for longer treatment periods (more than 6 months). However, to provide effective management of DMD, full knowledge will be required concerning the action of different treatments acting together, in order to establish an optimal combination of therapies, which will be necessary in a personalized manner, depending on patient's disease progress.

5. Conclusion

Exploratory data suggest that the intake of Ω-3LCPUFA (2.9 g/day) during 6 months likely slowed the progression of muscle loss, the decrease in fat mass, and the reduction of fasting insulin and insulin resistance in boys with DMD, which might exert an impact in terms of a better quality of life for these patients. Therefore, these exploratory results provide scientific background for the carrying out of a randomized trial focused of confirming the possible beneficial role of Ω-3LCPUFA on the previously mentioned alterations in boys with DMD with early muscle damage (without fibrosis).

Sources of support

This investigation was supported by Consejo Nacional de Ciencia y Tecnología-México (Grant#SALUD-2012-01-180058).

Conflict of Interest

None of the authors have conflicts of interest to declare.

CRediT authorship contribution statement

Maricela Rodríguez-Cruz: Conceptualization, Funding acquisition, Project administration, Resources, Writing - original draft, Writing - review & editing. **Salvador Atilano-Miguel:** Data curation, Formal analysis, Investigation, Methodology. **Lourdes Barbosa-Cortés:** Formal analysis, Investigation, Methodology. **Mariela Bernabé-García:** Investigation, Validation. **Tomas Almeida-Becerril:** Investigation, Methodology. **Alan Cárdenas-Conejo:** Methodology, Supervision, Writing - review & editing. **Oriana del Rocío Cruz-Guzmán:** Investigation, Methodology, Supervision. **Jorge Maldonado-Hernández:** Investigation, Methodology, Software.

Acknowledgments

We are grateful to the parents who authorized the participation of their children, the patients, in this study.

We thank the medical staff of the Clinical Genetic Pediatrics Hospital, CMN-Siglo XXI, IMSS National Medical Center, the "Dr. Gaudencio González Garza" General Hospital, and the CMN-La Raza IMSS National Medical Center for the clinical diagnosis of patients with DMD.

We are grateful to M. Sc. Marco Antonio Villaldama Soriano by the design of the Fig. 6.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2018.10.017.

References

[1] Birnkrant DJ, Bushby K, Bann CM, Apkon SD, Blackwell A, Brumbaugh D, et al. Diagnosis and management of Duchenne muscular dystrophy. Part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. Lancet Neurol 2018;17(3):251–67.
[2] Straub V, Campbell KP. Muscular dystrophies and the dystrophin-glycoprotein complex. Curr Opin Neurol 1997;10:168–75.
[3] Darras BT, Blattner P, Harper JF, Spiro AJ, Alter S, Francke U. Intragenic deletions in 21 Duchenne muscular dystrophy (DMD)/Becker muscular dystrophy (BMD) families studied with the dystrophin cDNA: location of breakpoints on Hind III and BglII exon-containing fragment maps, meiotic and mitotic origin of the mutations. Am J Hum Genet 1988;43:620–9.
[4] Gillard EF, Chamberlain JS, Murphy EG, Duff CL, Smith B, Burghes HM, et al. Molecular and phenotypic analysis of patients with deletions within the deletion-rich region of the Duchenne muscular dystrophy (*DMD*) gene. Am J Hum Genet 1989;45:507–20.
[5] Coral-Vázquez R, Arenas D, Cisneros B, Peñaloza L, Salamanca F, Kofman S, et al. Pattern of deletions of the dystrophin gene in Mexican Duchenne/Becker muscular dystrophy patients: the use of new designed primers for the analysis of the major deletion "hot spot" region. Am J Med Genet 1997;70:240–6.
[6] Berchtold MW, Brinkmeier H, Müntener M. Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. Physiol Rev 2000;80(3):1215–65.
[7] McDonald CM, Carter GT, Abresch RT, Widman L, Styne D, Warren N, et al. Body composition and water compartment in boys with Duchenne muscular dystrophy. Am J Phys Med Rehabil 2005;84:483–91.
[8] Zanardi MC, Tagliabue A, Orcesi S, Berardinelli A, Uggetti C, Pichiecchio A. Body composition and energy expenditure in Duchenne muscular dystrophy. Eur J Clin Nutr 2003;57:273–8.
[9] Willig TN, Carlier L, Legrand M, Riviere H, Navarro J. Nutritional assessment in Duchenne muscular dystrophy. Dev Med Child Neurol 1993;35:1074–82.
[10] Skalsky AJ, Han JJ, Abresch RT, Shin CS, McDonald CM. Assessment of regional body composition with dual-energy X-ray absorptiometry in Duchenne muscular dystrophy: correlation of regional lean mass and quantitative strength. Muscle Nerve 2009;39:647–51.
[11] Saure C, Caminiti C, Weglinski J, Pérez FD. Energy expenditure, body composition, and prevalence of metabolic disorders in patients with Duchenne muscular dystrophy. Diabetes Metab Syndr Clin Res Rev 2018;12:81–5.
[12] Rodríguez-Cruz M, Sánchez R, Escobar RE, Cruz-Guzmán OR, López-Alarcón M, Bernabé-García M, et al. Evidence of insulin resistance and other metabolic alterations in boys with Duchenne or Becker muscular dystrophy. Int J Endocrinol 2015;2015:867273. http://www.hindawi.com/journals/ije/aa/867273/.
[13] Weiss R, Kaufman FR. Metabolic complications of childhood obesity: identifying and mitigating the risk. Diabetes Care 2008;31:S310–6.
[14] Rodríguez-Cruz M, Cruz-Guzman OR, Escobar RE, López-Alarcón M. Leptin and metabolic syndrome in patients with Duchenne/Becker muscular dystrophy. Acta Neurol Scand 2016;133:253–60.
[15] García JJ, Rojas GG, León NM. Prevalencia de resistencia a la insulina y síndrome metabólico en niños obesos que acuden a la Clínica de Obesidad del Hospital Pediátrico de Sinaloa. Pediatr Mex 2010;12:18–22.
[16] Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Diabet Med 1998;15:539–53.
[17] Bianchi ML, Biggar D, Bushby K, Rogol AD, Rutter MM, Tseng B. Endocrine aspects of Duchenne muscular dystrophy. Neuromuscul Disord 2011;21:298–303.
[18] Couet C, Delarue J, Ritz P, Antoine JM, Lamisse F. Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults. Int J Obes 1997;21:637–43.
[19] Kabir M, Skurnik G, Naour N, Pechtner V, Meugnier E, Rome S, et al. Treatment for 2 mo with n-3 polyunsaturated fatty acids reduces adiposity and some atherogenic factors but does not improve insulin sensitivity in women with type 2 diabetes: a randomized controlled study. Am J Clin Nutr 2007;86:1670–9.
[20] Noreen EE, Sass MJ, Crowe ML, Pabon VA, Brandauer J, Averill LK. Effects of supplemental fish oil on resting metabolic rate, body composition, and salivary cortisol in healthy adults. J Int Soc Sports Nutr 2010;7:31.
[21] Rasic-Milutinovic Z, Perunicic G, Pljesa S, Gluvic Z, Sobajic S, Djuric I, et al. Effects of n-3 PUFAs supplementation on insulin resistance and inflammatory biomarkers in hemodialysis patients. Ren Fail 2007;29:321–9.
[22] López-Alarcón M, Martínez-Coronado A, Velarde-Castro O, Rendón-Macías E, Fernández J. Supplementation with n3 long chain polyunsaturated fatty acids diminishes insulin resistance of obese prepubertal and pubertal children in a synergistic manner with weight loss. Arch Med Res 2011;42:E502–8.
[23] Rodríguez-Cruz M, Cruz-Guzmán OR, Almeida-Becerril T, Solís-Serna D, Atilano-Miguel S, Sánchez-González JR, et al. Potential therapeutic impact of omega-3 long chain polyunsaturated fatty acids on inflammation markers in Duchenne muscular dystrophy: a double-blind, controlled randomized trial. Clin Nutr 2018;37(6):1840–51. https://doi.org/10.1016/j.clnu.2017.09.011.
[24] Vignos PJ, Spencer GE, Archibald KC. Management of progressive muscular dystrophy in childhood. JAMA 1963;184:89–96.
[25] Adams KM, Kohlmeier M, Powell M, Zeisel SH. Nutrition in medicine: nutrition education for medical students and residents. Nutr Clin Pract 2010;25:471–80.
[26] Rizzo AC, Goldberg TB, Silva CC, Kurokawa CS, Nunes HR, Corrente JE. Metabolic syndrome risk factors in overweight, obese, and extremely obese Brazilian adolescents. Nutr J 2013;12:19.
[27] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[28] Keskin M, Kurtoglu S, Kendirci M, Atabek M, Yazici C. Homeostasis model assessment is more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index for assessing insulin resistance among obese children and adolescents. Pediatrics 2005;115:500–3.
[29] Evershed RP. Gas chromatography of lipids. In: Hamilton RJ, Hamilton S, editors. Lipid analysis. A practical approach. Oxford, UK: Oxford University Press; 1992. p. 113–5.
[30] Ott Lyman. An introduction to statistical methods and data analysis. North Scituate, MA: Duxbury Press; 1993.
[31] Davidson ZE, Truby H. A review of nutrition in Duchenne muscular dystrophy. J Hum Nutr Diet 2009;22:383–93.
[32] Cao J, Schwichtenberg KA, Hanson NQ, Tsai MY. Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. Clin Chem 2006;52(12):2265–72.
[33] Gingras AA, White PJ, Chouinard PY, Julien P, Davis TA, Dombrowski L, et al. Long-chain omega-3 fatty acids regulate bovine whole-body protein metabolism by promoting muscle insulin signaling to the Akt-mTOR-S6K1 pathway and insulin sensitivity. J Physiol 2007;579:269–84.
[34] Robinson SM, Jameson KA, Batelaan F, Martin HJ, Syddall HE, Dennison EM, et al. Diet and its relationship with grip strength in community dwelling older men and women: the Hertfordshire Cohort Study. J Am Geriatr Soc 2008;56:84–90.
[35] Smith GI, Atherton P, Reeds DN, Mohammed BS, Rankin D, Rennie MJ, et al. Dietary omega-3 fatty acids supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. Am J Clin Nutr 2011;93:402–12.
[36] Ewaschuk JB, Almasud A, Mazurak VC. Role of n-3 fatty acids in muscle loss and myosteatosis. Appl Physiol Nutr Metabol 2014;39:654–62.
[37] Murphy RA, Mourtzakis M, Mazurak VC. N-3 polyunsaturated fatty acids: the potential role for supplementation in cancer. Curr Opin Clin Nutr Metab Care 2012;15:246–51.
[38] Pappalardo G, Almeida A, Ravasco P. Eicosapentaenoic acid in cancer improves body composition and modulates metabolism. Nutrition 2015;31:549–55.
[39] Fiaccavento R, Carotenuto F, Vecchini A, Binaglia L, Forte G, Capucci E, et al. An omega-3 fatty acid-enriched diet prevents skeletal muscle lesions in a hamster model of dystrophy. Am J Pathol 2010;177(5):2176–84.
[40] Rodríguez-Cruz M, Solís-Serna D. Nutrigenomic nutrigenomics of omega-3 fatty acids: regulators of the master transcription factors. Nutrition 2017;41:90–6.
[41] Dangart F, Chen Y, Gronowitz E, Dahlgren J, Friberg P, Strandvik B. High physiological omega-3 fatty acids supplementation affects muscle fatty acids composition and glucose and insulin homeostasis in obese adolescents. J Nutr Metab 2012:395757. https://doi.org/10.1155/2012/395757.
[42] Tishya A, Wren L, Bluml S, Tseng-Ong L, Gilsanz V. Three-point technique of fat quantification of muscle tissue as a marker of disease progression in Duchenne muscular dystrophy: preliminary study. Am J Roentgenol 2008;190:W8–12.