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# *Functional Training and Nutritional Education Improved Oxidative Stress in Children with Autism Spectrum Disorder- A Randomized Clinical Trial*

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## *Abstract*

*Covid-19 pandemic has affected individual lives in several aspects including physical activity and dietary pattern. Autism spectrum disorder (ASD) is a neuro-developmental disorder which is mostly known as social interaction impairment. It has been suggested that physical inactivity and proper diet, are common challenges among individuals with ASD. The purpose of this clinical trial was to investigate the effect of functional training along with online nutritional education on inflammatory biomarkers among children diagnosed with ASD. The participants were randomly divided into four groups including: 1) functional training, 2) online nutritional education, 3) training+ education and 4) control group. The intervention lasted for 8 weeks. The inflammatory biomarkers were assessed using anterior cubital blood vein samples, both before and after the study. Our results demostrated a significant increase in SOD ($p<0.001$), GPx ($p=0.02$) and CAT ($p<0.001$) level. However, there was no significant difference for LDH between the experimental groups($p=0.3$). In conclusion, functional training and online nutritional education can be considered as beneficial interventions for antioxidant enzyme but not for LDH, in children with ASD, which can be recommended for health status improvement.*

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## Introduction

The COVID-19 pandemic has had widespread consequences for population health, disproportionately affecting individuals with pre-existing health vulnerabilities and chronic conditions [1]. Public health restrictions implemented to mitigate viral transmission, including lockdowns and school closures, have substantially altered daily routines, physical activity patterns, and dietary behaviors, particularly among children [2,3]. These disruptions have raised concerns about the secondary health effects of the pandemic, especially in populations already at increased risk for adverse health outcomes [4,5].

Children with autism spectrum disorder (ASD) represent a particularly vulnerable group, as they are more likely to experience health-related complications associated with sedentary behavior, such as limited physical activity, and sub-optimal dietary patterns [6,7]. Such lifestyle factors may contribute to poor metabolic and physiological health and have been exacerbated during the COVID-19 pandemic due to reduced access to structured physical activity and support services [8]. Consequently, there has been growing interest in preventive strategies aimed at maintaining health and resilience in children with ASD through modifiable lifestyle interventions, including physical activity promotion and nutritional education [9,10].

Emerging evidence suggests that altered redox homeostasis, reduced antioxidant capacity, and increased oxidative stress are prevalent in children with ASD and may play a role in broader physiological dysregulation [11,12]. Oxidative stress has been proposed as a sensitive biomarker reflecting the cumulative impact of lifestyle, metabolic, and inflammatory factors, making it a potentially relevant outcome in preventive health research [13]. While antioxidant enzymes, serve as essential components of cellular defence against oxidative damage [14,15], their activity may be reduced under pathological conditions, either poor lifestyle habits including physical inactivity and nutritional challenges [16-18].

From a preventive medicine perspective, combined lifestyle interventions that integrate physical activity and nutritional education may offer a feasible approach to mitigating secondary health risks in vulnerable pediatric populations during public health crises. However, evidence evaluating the effectiveness of such combined interventions in children with ASD during the COVID-19 pandemic remains limited. Therefore, the aim of the present study was to investigate the effects of a combined functional exercise program and online nutritional education on oxidative stress markers in children with ASD during the COVID-19 lockdown period.

## Methodology

This study was a randomized-controlled clinical trial with pre-test and post-test assessment (Figure 1). The participants were 8-12 years with autism spectrum disorder, approved by the neurologist. The participants were selected by targeted selection and were randomly allocated to one of these experimental groups 1) functional exercise, 2) online nutritional education, 3) exercise+ education and 4) control group. Inclusion criteria for this study were not having any inhibition for exercise, no physical exercise experience within the past 6 month, and not reaching to puberty according to specialist approval. Participants were allowed to leave the study whenever they did not have tendency and the participant was excluded in case of absence for more than two sessions.All the study procedure was done under occupational therapist, physiotherapist and dietitian supervision and cooperation. The study protocols were approved by the ethics committee and were registered as a clinical trial at Iranian Registry of Clinical Trials with the IR.IAU.SRB.REC.1400.003 and IRCT20201211049678N1 identification numbers, respectively.

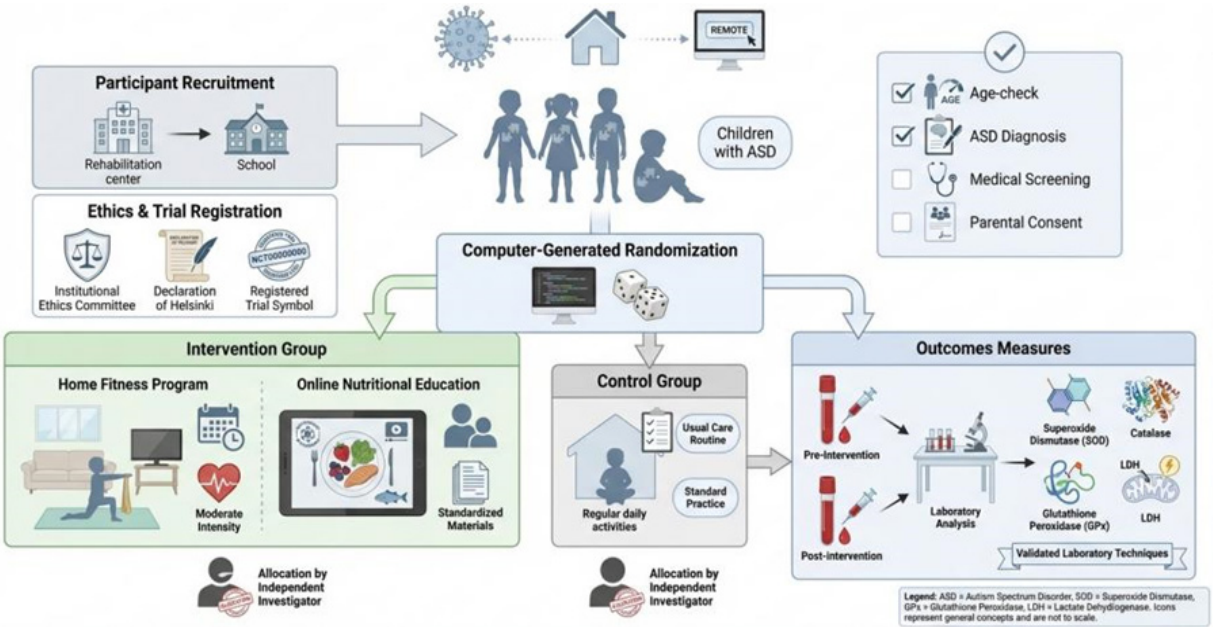


**Figure 1:** Randomized Clinical Trial Study Procedure.

## Clinical Screening and Pre-Assessment

Health file was created for each participant and previous biochemical laboratory data, medication, physical activity experience, 24-hour food recall and food frequency questionnaire (FFQ) data were collected [19]. Caregivers were instructed about how to complete FFQ prior to the study. All the medical history recording were completed by supervision of clinical dietitian. In order to simplify, home-based scales were used and were then transmitted to grams according to reference database and were calculated using N4 software [20]. Nutrition knowledge questionnaire (NKQ) was used to assess caregivers nutritional knowledge [21]. Children's physical activity questionnaire (CPAQ) was used to assess physical activity status in the participants before and after the intervention which assess physical activity level in 4 areas as following: 1) exercise activities, 2) leisure tome activities, 3) school-related activities such as walking to school and 4) other activities including: watching the TV, mental games, computer and internet use. Antioxidant enzymes including SOD, GPx, CAT and LDH, were assessed before and after the study. ZellBio kit was used for SOD, GPx and CAT. LDH enzyme level was measured using Pasr azmoon kit with 400 ml volume [22].

## Functional Exercise

The functional exercise program and the progression is illustrated in Figure 2. Functional exercise group participants, participated in functional exercise program for 8 weeks, 3 sessions per week and 45-60 minutes for each session. Each session included warm up, stand-sit with weights, going up and down the stairs, stretch movements and cool down under specialist supervision [23-25].

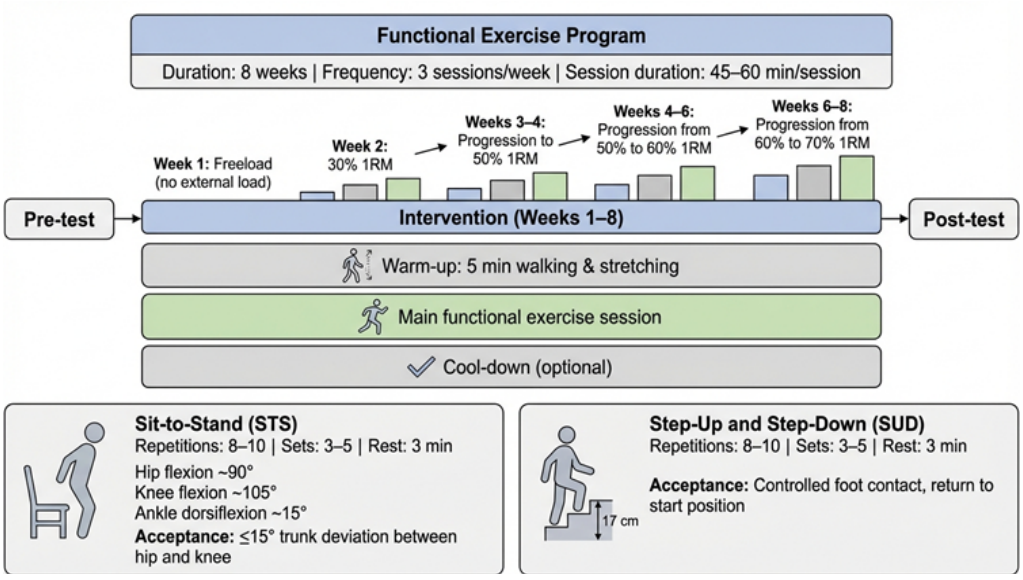


**Figure 2:** Functional Exercise Program and Progression

## Online Nutritional Education

Figure 3 shows online nutritional education program. Participants in education group participated in online nutritional education program for 8 weeks, 3 times per week for 30-15 minutes [26].Online nutrition educational program had targeted to improve caregivers nutritional knowledge in order to improve children's dietary pattern quality and included 4 areas according to NKQ approach as following: 1) nutritional advises, 2) food groups, 3) health food choices and 4) diet-related disease and weight management [26-29].

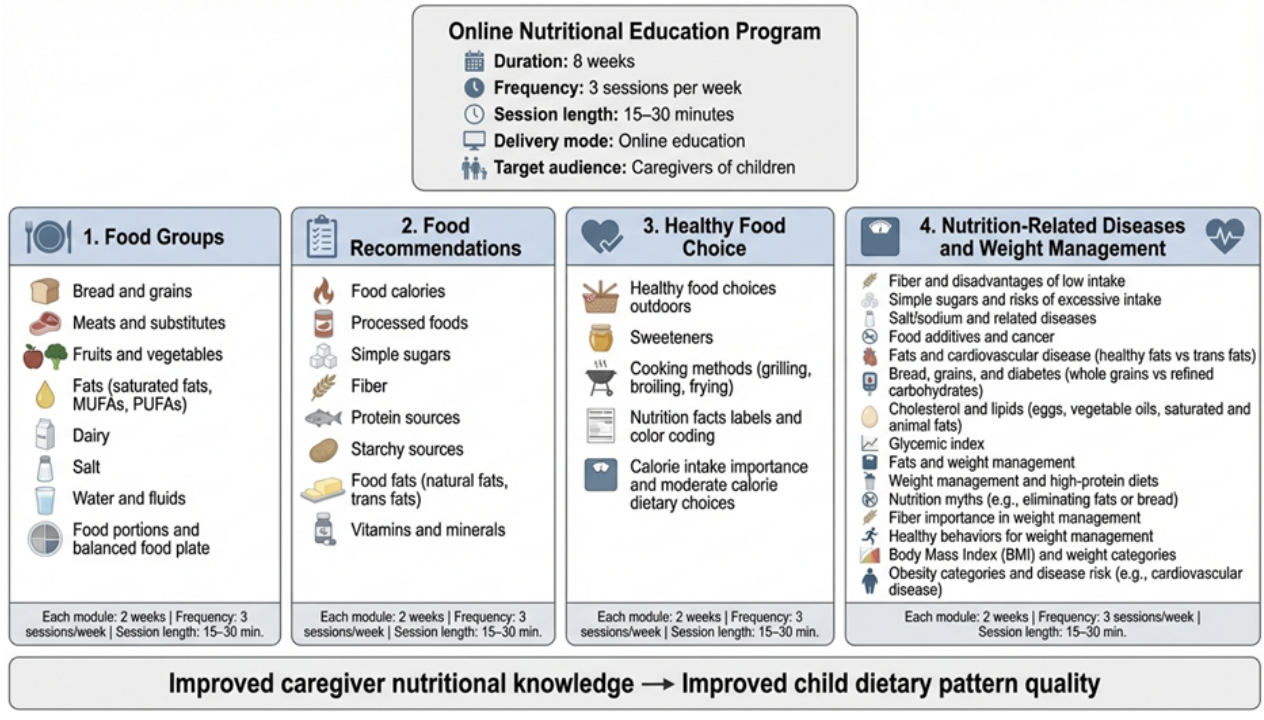


**Figure 3:** Online Nutritional Education Program

### Exercise+ Online Nutritional Education

Participants in exercise+ education group participated at functional exercise 3 session per week for 60-45 minutes and online nutritional education program 3 times per week for 30-15 minutes for 8 weeks.

## Results

### Participant Characteristics

Participants in the present study were boys aged 8–12 years with a confirmed diagnosis of autism spectrum disorder (ASD) made by a clinical specialist. Baseline comparisons indicated no significant differences among the study groups with respect to demographic and anthropometric characteristics, including age, body weight, and height (all $p > 0.05$), confirming group comparability prior to the intervention.

### Oxidative Stress Markers

### Superoxide Dismutase (SOD)

Baseline analysis showed no significant differences in SOD levels among the groups before the intervention ($p = 0.85$). Following the intervention period, a significant between-group difference was observed ($p < 0.001$). Compared with the control group, SOD levels increased significantly in the training group (mean increase = 7.89 units, SE = 2.20, $p = 0.001$), the online nutritional education group (mean increase = 20.94 units, SE = 2.21, $p < 0.001$), and the combined training plus education group (mean increase = 32.05 units, SE = 2.20, $p < 0.001$). Post-hoc LSD comparisons revealed significant differences between all intervention groups. SOD levels were significantly lower in the training group compared with the online education group (mean difference = 13.05 units, $p < 0.001$) and the combined intervention group (mean difference = 24.16 units, $p < 0.001$). In addition, SOD levels in the online education group were significantly lower than those observed in the combined training plus education group (mean difference = 11.11 units, $p < 0.001$).

### Catalase (CAT)

No significant differences in CAT activity were detected between groups at baseline ($p = 0.21$). However, a significant between-group difference emerged following the intervention ($p < 0.001$). Relative to the control group, CAT levels increased significantly in the training group (mean increase = 3.22 units, SE = 0.78, $p < 0.001$) and in the online education group (mean increase = 2.67 units, SE = 0.78, $p = 0.001$). No statistically significant change in CAT activity was observed in the combined training plus education group compared with the control group ($p = 0.09$). Post-hoc analysis indicated no significant difference between the training and online education groups ($p = 0.48$), nor between the online education and combined intervention groups ($p = 0.10$). A significant difference was observed between the training and combined training plus education groups ($p = 0.02$), with CAT levels being higher in the training group by 1.87 units.

### Glutathione Peroxidase (GPx)

At baseline, GPx levels did not differ significantly among groups ($p = 0.43$). After the intervention, a significant between-group effect was identified ($p = 0.02$). Compared with the control group, GPx activity increased significantly in the training group (mean increase = 0.03 units, SE = 0.01, $p = 0.01$), the online education group (mean increase = 0.03 units, SE = 0.01, $p = 0.005$), and the combined training plus education group ($p = 0.01$). LSD post-hoc comparisons showed no significant differences in GPx levels between the training and online education groups ($p = 0.73$), between the training and combined intervention groups ($p = 0.90$), or between the online education and combined intervention groups ($p = 0.82$).

### Lactate Dehydrogenase (LDH)

No significant differences in LDH activity were observed between groups either at baseline ($p = 0.96$) or following the intervention ($p = 0.30$).

## Discussion

The purpose of this study was to evaluate the effect of functional exercise and online nutritional education on oxidative stress biomarkers among children with ASD, when applied individually either in combination. Antioxidant enzymes were evaluated as indicators of oxidative stress and antioxidant capacity at baseline and following the completion of the intervention period. Previous epidemiological evidence suggests that the impact of exercise on inflammatory and oxidative stress biomarkers is strongly dependent on exercise intensity and workload.

Specifically, low to moderate exercise intensities may be insufficient to elicit meaningful reductions in inflammatory or oxidative biomarkers associated with

immune dysfunction [30]. In children with mental and intellectual disorders, sedentary behavior and inadequate nutritional habits are particularly prevalent and have been identified as major contributors to elevated oxidative stress levels in children with autism spectrum disorder [31].

Oxidative stress plays a critical role in disease development, and it has been suggested that interventions lasting at least eight weeks may lead to improvements in inflammatory markers, oxidative stress indices, and overall antioxidant capacity [32]. Superoxide dismutase (SOD) is considered the first line of antioxidant defense, catalyzing the dismutation of superoxide radicals, which are subsequently neutralized through the coordinated actions of glutathione peroxidase (GPx) and catalase (CAT), processes that are essential for maintaining redox homeostasis [33].

The findings of the present study demonstrated a significant increase in SOD levels across all intervention groups compared with the control group. Among the interventions, the combined functional training and online nutritional education program produced the greatest increase in SOD activity. Online nutritional education alone showed a moderate effect, whereas functional training alone resulted in the smallest, though still significant, improvement. These findings are noteworthy, given prior evidence indicating that eight weeks of endurance or resistance training can significantly enhance SOD activity [34]. The relatively smaller effect observed with functional training alone in the present study suggests that combining physical activity with nutritional education may exert a synergistic influence on antioxidant defense mechanisms, thereby producing superior outcomes.

Moderate exercise has been shown to transiently increase reactive oxygen species (ROS) production to an optimal level, which subsequently stimulates adaptive up-regulation of antioxidant enzymes, including GPx [35]. In line with this mechanism, GPx activity increased significantly in all intervention groups compared with the control group. However, no statistically significant differences were observed between the three intervention modalities, suggesting that functional training, online nutritional education, and their combination were similarly effective in enhancing GPx activity.

CAT activity also increased across all intervention groups from baseline to post-intervention. This improvement reached statistical significance in the functional training and online nutritional education groups when analyzed separately. In contrast, although CAT levels increased following the combined intervention, this change did not reach statistical significance. Previous research has reported beneficial effects of nutritional education on oxidative stress biomarkers, including CAT activity [36]. Interestingly, the present findings suggest that functional training may have a more pronounced influence on CAT activity than nutritional education alone.

Lactate dehydrogenase (LDH) levels decreased in all intervention groups; however, these reductions were not statistically significant. None of the interventions produced a meaningful effect on LDH activity. Despite the lack of significant findings in the current study, previous research has reported associations between nutritional status, dietary intake, and LDH activity [37]. Moreover, although the majority of studies support a positive role of exercise in improving oxidative stress, redox balance, and mitochondrial function, findings across the literature remain inconsistent. Some evidence indicates that longer-duration functional training programs, such as those lasting 12 weeks, may be required to induce significant changes in LDH and CAT activity, ultimately enhancing mitochondrial capacity and redox regulation [38].

While the present study demonstrated overall improvements in antioxidant enzyme activity following the interventions, it is important to note that not all studies have reported similar outcomes, highlighting the importance of intervention duration, intensity, and population characteristics when interpreting results [39].

Consistent with these findings, the present study demonstrated that eight weeks of functional training was the most effective intervention for improving upper limb strength when compared with online nutritional education alone and the combined intervention. Nevertheless, all three intervention groups exhibited significant improvements relative to baseline. The combined functional training and online nutritional education program ranked second in effectiveness, while online nutritional education alone showed the smallest improvement. A similar pattern was observed

for lower limb strength, with the exception that online nutritional education alone did not result in a statistically significant improvement. Notably, the combined intervention demonstrated greater efficacy for lower limb strength than functional training alone, suggesting an additive or synergistic effect.

In conclusion, functional training, online nutritional education, and their combination were all associated with improvements in oxidative stress–related biomarkers. Among the interventions, the combined approach generally produced the most favorable antioxidant responses. Despite these promising findings, further well-designed clinical trials with larger sample sizes, longer intervention durations, and controlled exercise intensities are needed to more precisely determine the independent and combined effects of physical training and nutritional education on oxidative stress outcomes in this population.

## Conflict of interests

The authors declare that there is no conflict of interest.

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