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Biochemical assessment of insulin and vitamin D levels in obese adolescents after diet and physical activity: A retrospective observational study

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Abstract

Study aim: Obesity is a serious public health problem that has spread over the past 40 years in industrialized countries. This condition can predispose to the onset of several chronic diseases for instance hyperlipidemia which is involved in multiple signaling pathways for bone homeostasis. There is a communication between adipose tissue and bone, which can regulate each other through feedback mechanisms including glucose consumption by bone, also regulating insulin levels. In our observational study, we analyzed the effects of low-impact training, particularly swimming, combined with a mediterranean diet on obese pre-adolescents.

Material and methods: Six-month of an observational study was performed involving twenty pre-adolescents aged between 8 and 12 years with diagnosed obesity with z-BMI >2, according to the World Health Organization guidelines.

Results: The assessment was carried out at the beginning of the intervention (T0) and at the end of treatment (T1). All participants were randomly assigned to either: the control group (CG) just followed the mediterranean diet whilst the experimental group (EG) over the mediterranean diet followed a planned physical activity.

The results showed statistically significant differences between T0 and T1 in both CG and EG, especially concerning $1,25(OH)_2D$ and insulin levels. However, the differences were more impressive in EG ($1,25(OH)_2D$ 9.27 vs 25.64; Insulin 29.31 vs 12.66) compared with CG ($1,25(OH)_2D$ 8.7 vs 13.7; Insulin 28.45 vs 22.76).

Conclusions: In conclusion, these results showed the importance of diet and low-impact exercise intervention to improve pre-adolescent's health especially those with obesity.

Keywords: Physical activity – $1,25(OH)_2D$ – Swimming – Obesity – Insulin – Exercise

Introduction

Obesity is a major public health problem that has been rampant over the past 40 years in industrialized and developing countries [28]. It is determined by the interaction of genetic and environmental factors and mainly by insufficient physical activity (PA) and improper nutrition [26]. High-fat diets are known to alter lipids serum levels, cytokines, hormonal factors and other markers that can promote changes in the quality and quantity of bone microarchitecture and strength. Conversely, it would be appropriate to prefer diets suitable for health such as the Mediterranean diet [2]. In this regard, a hyperlipidaemic environment involves multiple signaling pathways of bone homeostasis such as vitamin D (VitD) [27]. In the obese, a reduced bioavailability of vitamin D has been found due to its deposition in body fat compartments. Furthermore, visceral fat can cause metabolic abnormalities by secreting inflammatory adipokines that induce insulin resistance/diabetes and metabolic abnormalities of vitamin D [12]. There is evidence that supports crosstalk

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between adipose tissue and bone, which regulate each other through a feedback mechanism which originates from bone marrow mesenchymal stem cells (BMSCs) that can differentiate into osteoblasts and adipocytes [17, 18]. One of these concerns the consumption of glucose by the bone, which occurs preferentially through aerobic glycolysis, also regulating insulin levels [10]. According to a recent survey, from the NCD-RisC (Non-Communicable Disease Risk Factor Collaboration) study, the prevalence of paediatric obesity has increased considerably from 4% in 1975 to 18% in 2016 [5]. This is associated with an increase in comorbidities, previously framed in the adult population, such as type 2 diabetes mellitus, non-alcoholic hepatic steatosis (NAFLD), obstructive sleep apnoea (OSA) and dyslipidaemia. However, the psychosocial aspect should not be overlooked, especially during adolescence, which in the context of obesity given to unhealthy eating habits, has led to numerous cases of bulimia nervosa, uncontrolled eating disorder or night eating syndrome [14]. In the developmental age, the diagnosis of obesity is performed through tables of percentiles which consider height, weight and the percentage of body fat, which varies according to gender and age [28]. To counter the further spread of this phenomenon, the World Health Organisation (WHO) guidelines, recommend that pre-adolescents and adolescents aged between 5 and 17 years, should perform at least 60 minutes of moderate to vigorous intensity PA, which can be achieved by performing activities in several short periods during the day [3]. These suggestions include playing sports, active transport, recreation and physical education, as well as planned exercise. Marson et al., in a meta-analysis published in 2016, included a randomised clinical trial lasting at least 6 weeks and concluded that aerobic exercise training is the most suitable activity modality in reducing fasting insulin levels and Homeostatic Model Assessment (HOMA), an evaluation index aimed at identifying insulin resistance and/or metabolic syndrome in pre-adolescents with obesity [20, 30]. Similarly, resistance training seems to improve insulin action and glycoregulation, avoiding the loss of muscle mass [6, 16]. However, there is a lack of studies in the literature that have focused on issues related to the participation of pre-adolescents with obesity in various sports [7]. One explanation could be that excess weight impairs motor skills and triggers negative feelings and low self-esteem, leading to the promotion of sedentary habits [4]. Among the few papers published in this field is a study reported by Dana L. et al. that analysed team sports as an after-school activity in pre-adolescent obesity. The results were encouraging, seeing it as a valuable opportunity to both breakdown barriers to playing sports and reduce body weight [22]. The PA intervention coupled with nutritional advice produced significant results in terms of body weight reduction at the end of a school year [26]. So far, many studies have confirmed the significant correlation between VitD deficiency and obesity in pre-adolescents [19]. In light of the above, it is clear that in order to intervene in this public health problem, it is necessary to act through a multidisciplinary approach in which PA and nutrition are the pivotal factors. The aim of the study was to provide guidelines to counteract the possible consequences of obesity in pre-adolescents. These certainly include the development and functionality of the musculoskeletal system, such as the early onset of joint pain, incorrect postures, abnormal loads on certain joints, and alterations in bone growth. Swimming is proposed as an excellent intervention strategy to achieve the dual objective of weight loss and protection of the health and development of the subject at a young age. Obviously that should be associated with a correct eating style such as that indicated by the Mediterranean diet.

Materials and methods

Participants

We conducted a 6-month retrospective observational study including 21 pre-adolescents aged from 8 to 12 years (height of CG 143 ± 7.89 cm vs EG 142 ± 13.2 cm), (CG 6 females and 4 males; EG 6 females and 5 males) with obesity defined according to World Health Organization (WHO) standardized values for the BMI. In particular, this parameter, z-BMI, is used to monitor the changes that occurred in subjects by age and gender (>2 z-score for obesity; >1 and ≤2 for overweight; ≥–2 and ≤1 for normal weight). Before starting, the parents and their pre-adolescents filled out a questionnaire to gather information about the eating and lifestyle habits by the family paediatrician. Written informed consent to participate in this study was provided by the participant's legal guardian/next of kin. The anthropometric data (height and weight) was collected from nutritionists specialized in pediatric and/or childhood obesity using a wall-mounted stadiometer and a calibrated scale, respectively. Body composition analysis was performed using the Akern Bioelectrical Impedance Analysis (BIA) 101 Physiological Data Analyzer (AKERN SRL, RJL Systems, Detroit, USA). During the intervention period, all participants were monitored periodically every forty-five days.

Experimental design

The pre-adolescents involved in the study had a z-BMI >2 and were randomly assigned in two groups: 10 pre-adolescents in a control group who were following a structured nutritional therapy program (CG) and 11 pre-adolescents in an experimental group who were following a structured nutritional therapy program combined with swimming performed three times a week (EG). The swimming session took place indoors because a prolonged activity under the sun could have provided an additional treatment on VitD, so the EG had swimming sessions 3 times a week in an indoor pool.

At the beginning (T0), a careful anamnesis of each participant was carried out by the specialists, to detect lifestyle, eating habits, hours of rest, the hemathochemical parameters and basic swimming skills, to structure an adequate training plan. As concerned the hemathological tests results, were assessed a deficiency of VitD, through the analysis of the 1,25 OH-D levels. It is generally used to determine the levels of this metabolite, which originates from the metabolism of cholecalciferol first in the liver and then in the kidney, as it is the most active form that plays an essential role in the active and efficient absorption of calcium and phosphorus. It is therefore identified as a marker of the onset of diseases and conditions affecting normal calcium and phosphorous metabolism. Starting from this evidence, all the participants start to intake daily supplementation with 400 IU of cholecalciferol twice a day. All subjects showed basic aquatic development consistent with the ability to focus on the environment, adaptation, respiration, and the ability to float and move independently. The EG subjects practiced swimming three times a week, with each swimming session lasting one hour. The training plan was structured in two different sessions called A and B, which alternated on three midweek days, for example, A-B-A; every single training session was divided into three phases.

Session A was structured as follows:

- The warm-up phase (10 min) was carried out outside the pool with muscle activation exercises, joint mobility and motor coordination (eg rotation of shoulders, neck, knees, wrists and ankles);
- The central phase (40 min) in which the training took place and was composed of 25 min of exercises aimed at improving the motor patterns of basic swimming, such as buoyancy, maintaining water posture, and developing safe water entry, including diving, running 10 meters back and forth, turning, and knowledge of water safety; 15 min for activities to strengthen swimming skills, through a series of games played in groups and to allow pre-adolescents to socialize with each other;
- A cool-down and stretching phase (10 min), in which the activities were carried out outside of the water.

Session B was structured as follows:

- The warm-up phase (10 min) similar to the session A;
- The central phase (40 min) consists of 25 min of exercises aimed at improving backstroke and crawling and preparing the first exercises for symmetrical breaststroke and dolphin swimming; 15 min for activities to enhance swimming skills, through a series of games played in groups and to allow pre-adolescents to socialize with each other;
- A cool-down and stretching phase (10 min each) similar to the session A.

Statistical analysis

Statistical analysis was performed with Jamovi (The jamovi project (2021). Jamovi (Version 1.8.0.1). Retrieved from https://www.jamovi.org). A Shapiro-Wilks test was performed to identify the normality of the distribution of all parameters. Parametric and non-parametric evaluation was adopted when appropriate. To evaluate the differences between the T0 and T1 measurements of the same group (CG or EG), a paired-data t-test was used for the first case and an independent t-test for the second case. In addition, effect size was performed to describe the strength of the relationship between the variables.

Nutritional evaluations

Nutritional assessments

The usual food consumption in the previous months was evaluated with the questionnaire sent before the visit from the nutritionist. The adequacy of the intake of macronutrients and micronutrients (iron, zinc, VitD and calcium) was assessed based on the reference levels of nutrient and energy intake for the Italian population (LARN) published by the SINU 2014 review. Dietary suitability was assessed by analysing the critical issues that emerged from the questionnaires on eating habits and meal frequency. A nutritional plan was subsequently drawn up by specialized personnel.

Recorded total energy intake, also adjusted for body mass, was not significantly different between EG and CG. Similarly, was observed comparable percentages of macronutrients (fat, saturated fat, carbohydrate, and protein) in total energy intake for EG and CG. For both groups, was administered a Mediterranean diet with 52% Carbs of which monosaccarides <10%, proteins <15% (1g/kg) and fat <34% of which satured <10%.

Daily calorie requirements and distribution

For the diets of the obese pre-adolescent, the total calories obtained from the formula, must be reduced by 20%.

Table 1. Calculation for the allocation of daily calories of obese children

Body weight [kg]	Calories
0–10	0 + (P.I. – 0) × 100
11–20	1000 + (P.I.) – 10) × 50
21–95	1500 + (P.I. – 20) × 20

P.I.; ideal weight at the 50th percentile relative to the child's actual height.

Table 2. Calorie breakdown of daily requirement amounts

Breakfast	15–20%
Snack	5%
Lunch	35–45%
Snack	5–10%
Dinner	30–35%

Results

The statistical analysis of the assessment performed at T0 did not show a significant difference between the two groups (CG and EG) (table 3).

Nevertheless, after six months of intervention (T1) major changes were found between the two groups (table 4) overall as regards 1,25(OH)$_2$D and insulin (respectively of 1,25(OH)$_2$D 13.70 ± 2.11 ng/ml in CG vs 25.64 ± 4.24 ng/ml in EG and insulin 22.76 ± 10.22 UI in CG vs 12.66 ± 9.27 UI in EG).

As regards the analysis of the experimental group between the time T0 and T1, important differences were found for all parameters analyzed; in particular the reduction of weight (55.25 ± 10.38 Kg vs 50.42 ± 10.21 kg, $p < 0.002$), the increase in free fat mass (FFM) (33.70 ± 8.19 Kg vs 35.89 ± 8.59 kg, $p < 0.007$), a reduction of fat mass (FM) (21.55 ± 4.95 kg vs 15.06 ± 5.51 kg, $p < 0.001$), 1,25(OH)$_2$D (8.70 ± 0.67 ng/ml vs 13.70 ± 2.11 ng/ml, $p < 0.001$) and insulin (29.31 ± 8.78 UI vs 12.66 ± 9.27 UI, $p < 0.001$) (table 4).

After six months, the CG also showed statistically significant changes in: weight (54.91 ± 21.45 kg vs 53.51 ± 21.44 kg, $p < 0.007$), FM (20.66 ± 6.51 kg vs 18.09 ± 7.77 kg, $p < 0.01$), 1,25(OH)$_2$D (8.70 ± 0.67 ng/ml vs 13.70 ± 2.11 ng/ml, $p < 0.001$) and insulin (28.45 ± 10.76

Table 3. Anthropometrical and hemathochemical differences between CG and EG at the baseline (T0)

	T0			
	CG	EG		
Variables	Mean ± SD	Mean ± SD	p value	ES
Weight [kg]	54.91 ± 21.45	55.25 ± 10.38	0.964	0.020
FFM [kg]	34.25 ± 15.82	33.70 ± 8.19	0.920	0.044
TBW [%]	26.34 ± 11.15	26.67 ± 6.43	0.933	0.037
FM [kg]	20.66 ± 6.51	21.55 ± 4.95	0.728	0.154
1,25(OH)$_2$D [ng/ml]	8.70 ± 0.67	9.27 ± 1.73	0.341	0.426
Insulin [UI]	28 45 ± 10.76	29.31 ± 8.78	0.843	0.087

CG control group; EG experimental group; FFM free fat mass; TBW total body water; FM fat mass; 1,25(OH)2D; 1,25-dihydroxyvitamin D – EF effect size.

Table 4. Anthropometrical and hemathochemical differences between CG and EG at the baseline (T1)

	Control group				Experimental group			
	T0	T1			T0	T1		
Variables	Mean ± SD	Mean ± SD	p value	ES	Mean ± SD	Mean ± SD	p value	ES
Weight [kg]	54.91 ± 21.45	53.51 ± 21.44**	0.007	1.088	55.25 ± 10.38	50.42 ± 10.21**	0.002	1.242
FFM [kg]	34.25 ± 15.82	35.42 ± 14.82	0.080	0.624	33.70 ± 8.19	35.89 ± 8.59**	0.007	1.031
TBW [%]	26.34 ± 11.15	27 54 ± 10.21	0.052	0.707	26.67 ± 6.43	29.25 ± 7.69*	0.011	0.945
FM [kg]	20.66 ± 6.51	18.09 ± 7.77**	0.010	1.033	21.55 ± 4.95	15.06 ± 5.51***	<0.001	1.608
1,25(OH)$_2$D [ng/ml]	8.70 ± 0.67	13.70 ± 2.11***	<0.001	4.27	9.27 ± 1.73	25.64 ± 4.24***	<0.001	4.27
Insulin [UI]	28.45 ± 10.76	22.76 ± 10.22***	<0.001	2.13	29.31 ± 8.78	12.66 ± 9.27***	<0.001	2.13

*** – statistically significant difference, $p \leq 0.001$; ** – statistically significant difference, $p \leq 0.01$; * – statistically significant difference, $p \leq 0.05$; CG control group; FFM free fat mass; TBW total body water; FM fat mass; 1,25(OH)$_2$D; 1,25-dihydroxyvitamin D – ES effect size.

UI vs 22.76 ± 10.22 UI, $p < 0.001$) but these changes were not as large as those in the EG.

Whilst there were no statistically significant changes in FFM between T0 and T1 (34.25 ± 15.82 kg vs 35.42 ± 14.82 kg, $p > 0.05$).

Discussion

Given the complexity of the disease of obesity, research in the field suggests that multidisciplinary action needs to be taken in which physical activity and nutrition are among the most important actions to be performed.

This retrospective observational study aimed to investigate the effects of a swimming programme combined with a controlled diet in obese pre-adolescents. Based on the obtained results, this strategy appears to be successful. In particular, greater benefits were observed in the EG in terms of reducing FM, and insulin levels and increasing $1,25(OH)_2D$ levels to enough of a level that it comes within physiological ranges compared with the CG. The relationship between $1,25(OH)_2D$ deficiency and pediatric obesity has been widely discussed in numerous studies collected in the meta-analysis carried out by Fiamenghi and Mello [9]. It is already known that over this deficiency, excess weight, as well as adipose tissue, can be predisposing factors to the onset of insulin resistance or another obesity-related dysmetabolic state that could have a negative impact on bone health not only in terms of bone mineral density but also as mechanical overload [11, 23]. There are many evidences that demonstrated the health benefits of cholecalciferol supplementation. Daneels et al., demonstrated that gestational supplementation could prevent adverse outcomes and have a positive influence on the length of telomeres that confer chromosomal stability [8]. This is why several international scientific societies [12, 21, 32] agree to recommend cholecalciferol supplementation during the first year of life, believing that even 400 IU/day are safe and effective for preventing rickets [25]. Regarding interventions based on sports such as swimming, which has a low impact on fragile obese subjects, no examples have been reported in the literature so far. The difficulty in suggesting sports practices to counteract this condition lies precisely in the excess weight that impairs motor performance and development with negative repercussions also on the sense of self-efficacy and self-esteem and thus on psychological well-being [24, 31]. Recently, Trecodi et al. showed that there is a significant correlation between physical fitness and anthropometric parameters [29]. These results are confirmed by previous studies that have shown that pre-adolescents with a higher body mass index have reduced motor performance compared to normal-weight peers [1, 13]. Swimming, due to its low-impact musculoskeletal impact, could be a safe practice for obese pre-adolescents. Only one study from Kolieb et al. [15] investigated the effects of cholecalciferol supplementation combined with swimming practice in an obese mouse model; the results showed that swimming training for 6 weeks and cholecalciferol supplementation of 500 IU/kg attenuated body weight gain, improved lipid and glycemic profiles, and reduced serum insulin levels. In the experimental study we observed, significant differences were shown between the obese pre-adolescents enrolled and who were randomly assigned to one of two groups: CGs treated only with a planned diet and EGs who in addition to diet also did physical activity. Measurements taken at time T0 and T1 showed improvements regarding body recomposition and in particular on the levels of some hematochemical parameters such as $1,25(OH)_2D$ and insulin, demonstrating that a well-structured nutritional plan induces an improvement in the body structure of the pre-adolescents. In addition, if this is associated with physical activity such as swimming, the improvements are found to be significantly greater, demonstrating the importance of exercise on the quality of life of each subject.

Although this study produced encouraging results, it has limitations due to the small sample selected, and therefore future studies should deepen current knowledge by increasing the sample size and extending the duration of the intervention.

Conclusion

An optimal nutritional plan in pre-adolescents is known to lead to improved body parameters. The association of an amateur sport such as swimming, would seem to lead to a significant increase in these parameters, as well as VitD and the general health condition of the pre-adolescents. Therefore, on the basis of our observations, we can report that although the need to intervene at several levels to fight against obesity in pre-adolescents by involving the family network, school and in general doctors and health professionals has been recognised, there still remains a field to be explored, especially that concerning sports practice, both individual and team, and dietary program [22]. This observational study demonstrates that sports, and in particular swimming combined with a Mediterranean diet, may be the best strategy at least at an early stage when subjects need to lose weight. At the same time this avoids health impairment of the developing musculoskeletal system, causing also an improvement in some parameters which are sometimes affected during weight loss, such as lean mass.

Conflict of interest: Authors state no conflict of interest.

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